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REGIONAL CEREBRAL BLOOD FLOW AUTOREGULATION AND CEREBRAL VASOSPASM IN MONKEYS WITH SUBARACHNOID HEMORRHAGE

by

© DONALD PAUL JOSEPH BOISVERT

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled REGIONAL CEREBRAL BLOOD FLOW AUTOREGULATION AND CEREBRAL VASOSPASM IN MONKEYS WITH SUBARACHNOID HEMORRHAGE submitted by DONALD PAUL JOSEPH BOISVERT in partial fulfilment of the requirements for the degree of Doctor of Philosophy in EXPERIMENTAL SURGERY.

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External Examiner

DATE July 9th, 1976.

ABSTRACT

Regional cerebral blood flow measurements (intra-arterial ^{133}Xenon technique) and cerebral angiography were performed in two series of macaque monkeys.

Cerebral blood flow autoregulatory and arterial caliber responses to increases in mean arterial blood pressure (MaBP) were studied before and during the acute period following induced subarachnoid hemorrhage or mock subarachnoid hemorrhage (subarachnoid injection of artificial cerebrospinal fluid) in rhesus monkeys.

A comparative study of the acute effects of subarachnoid injections of artificial cerebrospinal fluid, serotonin and blood on regional cerebral blood flow, cerebral arterial caliber, neurological status and cerebral vessel ultrastructure was carried out in cynmolgous monkeys.

Regional cerebral blood flow autoregulatory responses to increases in MaBP were impaired following subarachnoid hemorrhage. Autoregulatory impairment was associated with loss of cerebral arterial vasomotor tone. Mock subarachnoid hemorrhage had no effect on autoregulation or vasomotor tone.

Subarachnoid injection of blood in cynmolgous monkeys caused a decrease in cerebral blood flow lasting less than one hour and moderate vasospasm which lasted at least three hours. In contrast, subarachnoid injection of artificial cerebrospinal fluid had no effect on these parameters. Subarachnoid injection of a serotonin solution at physiological concentrations (as determined by in-vitro bioassay) was also made without effect on cerebral blood flow or arterial caliber. Higher (10x) serotonin concentrations caused a cerebral blood flow response similar to that obtained with blood. However, the cerebral vasospasm induced was of shorter duration than that obtained with blood.

No significant neurological effect of these subarachnoid injections was observed during the 16 to 18 hour period following reversal of anesthesia. Animals with moderate vasospasm demonstrated no ultrastructural changes in cerebral arteries, arterioles and capillaries.

These results support the following conclusions:

(1) Cerebral flow regulatory mechanisms play an important role in determining cerebral perfusion status following subarachnoid hemorrhage.

(2) Moderate vasospasm does not impair cerebral perfusion in the presence of normal cerebral perfusion pressure and arterial blood gases.

(3) Unphysiologically high concentrations of serotonin are required to produce significant effects on the cerebral circulation.

(4) Development of post-SAH cerebral edema is at least partly due to the duration of intracranial hypertension which occurs during hemorrhage from a ruptured cerebral aneurysm.

PREFACE

It is generally recognized that neurological deterioration in patients with subarachnoid hemorrhage (SAH) is precipitated by the development of inadequate cerebral perfusion. The pathophysiological effects of SAH which lead to cerebral ischemia and infarction, however, have not been fully elucidated. Cerebral vasospasm is often present following SAH and appears to be more severe in patients with poor neurological status. This association may also be present in patients who deteriorate following successful surgical isolation of the aneurysm from the cerebral circulation. On the basis of these observations cerebral arterial spasm has been implicated as the principal cause of morbidity and mortality after aneurysmal rupture. However, severe vasospasm may be present in the absence of neurological deficit and conversely may be absent in the presence of neurological deficit. These observations indicate that other factors can produce critically low levels of cerebral perfusion, particularly in the presence of severe cerebral vasospasm.

In spite of numerous clinical and experimental studies, the etiology of cerebral vasospasm remains obscure. At present, the bulk of accumulated evidence points towards a chemical vasoconstrictive agent which is liberated from the blood present in the subarachnoid space. Many such agents have been identified in blood (e.g. serotonin,prostaglandins, norepinephrine) and each has its proponents as the agent causing cerebral vasospasm in man. However, recent evidence appears to be heavily weighted in favour of serotonin as the major spasmogenic agent.

The purpose of the present work was to investigate the pathophysiological effects of SAH on the cerebral circulation in a primate model, and to assess the role of serotonin in the pathogenesis of one of

these effects i.e. cerebral vasospasm.

Two separate studies are presented here: the first study concerns the acute effect of SAH on cerebral blood flow autoregulatory responses and cerebral vasomotor tone; the second study was an attempt to clarify the acute effects of SAH on cerebral blood flow, arterial caliber, neurological status and cerebral vessel ultrastructure and to compare these effects with those obtained when serotonin rather than blood is injected into the subarachnoid space. The major portion of each study consisted of cerebral blood flow measurements and angiographical evaluation of cerebral arteries following induced subarachnoid hemorrhage in macaque monkeys.

In this thesis is presented a brief review of relevant concepts and investigations pertaining to the physiology of cerebral blood flow regulation and the pathophysiology of SAH. In the latter, emphasis is placed on investigations dealing primarily with serotonin-induced cerebral vasospasm. Following this, a description of the methodology and instrumentation employed in the present studies is provided. The specific objectives, experimental designs, results and discussions for each of the two studies are subsequently presented separately. This format was adopted because of signficiant differences which existed between the two studies regarding objectives and experimental designs. Finally, the conclusions derived from this work are summarized.

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CHAPTER ONE

THE PHYSIOLOGY OF CEREBRAL BLOOD FLOW REGULATION

I. INTRODUCTION

Under normal physiological conditions the rate of blood flow in cerebral tissue is determine primarily by the relationship between perfusion pressure (CPP) and cerebrovascular resistance (CVR):

$$\text{CBF (ml/100g/min)} = \frac{\text{CPP (mmHg)}}{\text{CVR (mmHg/ml/100g/min)}}$$

The difference between mean arterial blood pressure (MaBP) and intracranial pressure (ICP) may be used as an index of CPP. Rowan et al (1) have demonstrated the validity of this definition of CPP over a wide range of ICP.

Factors which normally influence CBF do so primarily through their effect on cerebral vessel diameter and tone, thus inducing alterations in CVR; this includes CPP which itself affects CVR. Moderate variations in CPP, whether induced by changes in MaBP or ICP, are normally compensated for by appropriate changes in CVR. As a result, CBF remains relatively constant. The term 'autoregulation' is commonly used to describe this regulatory process.

The factors considered important for the normal regulation of CBF are discussed below.

II. CHEMICAL FACTORS

A. Arterial Carbon Dioxide

Early qualitative studies clearly demonstrated that arterial carbon dioxide ($PaCO_2$) levels constitute the most potent stimulus for changes in the cerebral circulation. (2,3) The apparent dominance of this factor

in controlling cerebral blood flow has been further confirmed and clarified using quantitative CBF measurement techniques (4,5).

In man, inhalation of 5 percent CO_2 raises CBF by 50-60 percent and 7 percent CO_2 is accompanied by a 100 percent increase in CBF, while reduction in $PaCO_2$ from 45 to 26 mm Hg decreases CBF by 35 percent (6). Reivich (7) measured CBF in rhesus monkeys at $PaCO_2$ levels ranging from 5 to 418 mm Hg and found that CBF response varied from a minimum flow of 45 percent of normal to a maximum flow of 240 percent of normal. Maximum sensitivity of CBF to change in $PaCO_2$ was calculated and found to be 1.11 ml/100g/min per mm Hg $PaCO_2$. In a similar study in dogs by Harper and Glass (8), CBF changed 2.5 to 3 percent for every 1 mm Hg change in arterial $PaCO_2$. This degree of CO_2 reactivity was present over a range of $PaCO_2$ values from 30-70 mm Hg. Similar values for CO_2 reactivity have been reported for cats (9), monkeys (10), baboons (11) and man (12). In these investigations, $PaCO_2$ values below 20 mm Hg or above 80 mm Hg generally have been found to exert little or no further effect on CBF

Direct observations of pial arteries and arterioles have confirmed that the effect of CO_2 on CBF is mediated through changes in vessel diameter (9,13,14). In the cat quantitative measurements of pial artery diameter responses to changes in $PaCO_2$ have revealed that a gradient of sensitivity exists between small and large pial arteries (13). In response to the same increase in $PaCO_2$, smaller vessels (diameter 13 to 40 μ) increased in diameter by approximately 48 percent whereas larger vessels (diameter 41 to 90 μ) increased by only 30 percent. A similar study subsequently carried out by Gotoh et al. (14) produced almost identical results. The occurence of maximal sensitivity to CO_2 at the pre-capillary arteriolar level suggests that the CO_2 produced during cerebral metabolism

has the potential to regulate CBF in accordance with local metabolic activity.

The specific mechanism for the effect of CO_2 on the cerebral circulation is a subject of considerable controversy. It is well established that an acute rise in $PaCO_2$ results in increased CBF, cerebral vasodilation, decreased CVR and increase in cerebral blood volume (5). Low $PaCO_2$ has the opposite effect. However, during sustained alterations in $PaCO_2$ in humans under a variety of conditions (15,16,17) CBF fails to correlate with $PaCO_2$ values. In these studies the pH of the perivascular fluid has been found to reflect changes in CBF and CVR much more consistently than arterial CO_2 tension. This has led to the hypothesis that the pH of the extracellular fluid of arteriolar smooth muscle is the common agent through which tissue and blood CO_2 control cerebrovascular tone (16). This hypothesis is supported by other experiments, in particular the work of Betz (18), Lassen et al. (19) and Kuschinsky et al. (20). In anesthetized cats, Betz demonstrated that cortical extracellular pH was more closely correlated with cortical vascular resistance than $PaCO_2$, PaO_2 or arterial pH during induced changes of the acid-base equilibrium of the brain. Lassen et al. and Kuschinsky et al. utilized a microapplication technique to alter the composition of the fluid surrounding pial arteries and arterioles in cats. This method allows observation of local vessel responses to changes in pH while $PaCO_2$ remains constant. In both studies, vasodilation and vasoconstriction occurred in response to lowering and raising the pH of the perivascular fluid. The conclusion was that extravascular pH is a major regulator of CVR and therefore CBF.

Results of other experiments in which alterations in brain pH failed

to induce changes in cortical flow (21,22) are at variance with the interpretation of the above studies. Moreover, the speed of the cerebral vessel response to alterations in CO_2 and the rapidity with which CO_2 enters cells (in comparison with H+) suggests a more direct effect of CO_2on the cerebral vessels (23). On this basis, Gotoh et al. (24), McDowall and Harper (21) and Shinohara (22) have suggested the intracellular pH of arteriolar smooth muscle cells is the dominant factor in the control of CBF.

The mechanism through which the intra- or extracellular H+ affects the cerebral circulation is unclear. However, the excitatory state of the smooth muscle membrane is clearly important in determining the ability of the smooth muscle cell to respond to vasodilator or vasoconstrictive stimuli. This has been recently ascertained by the experiments of Kuschinsky (20) and Betz (25) in which pial artery responses to H+ were shown to be markedly dependent upon local Ca++ and K+ concentrations. Other indirect evidence which may be offered in this regard is the fact that conditions which decrease CBF, cerebral metabolic activity, and presumably smooth muscle reactivity, diminish or abolish the response of the cerebral circulation to alterations in $PaCO_2$ (26,27,28).

B. Arterial Oxygen

The cerebral circulatory effects of alterations in arterial oxygen (PaO_2) over a wide range are modest and in the opposite direction when compared to CO_2 induced changes. Direct observation of pial vessels reveals a mild vasoconstrictor response to high oxygen tensions and a vasodilator response to low oxygen tension (29,30). Cerebral blood flow measurements in man by Kety and Schmidt (6) have shown corresponding changes in cerebral perfusion rates with a 13% decrease in mean flow with

inhalation of 85 - 100% O_2 and a 35% flow increase with inhalation of 10% O_2.

Although, the CBF effects due to changes in CO_2 tension are much more pronounced than those due to changes in oxygen tension, the decrease in PaO_2 to hypoxic (PaO_2 less than 45 - 50 mm Hg) levels which occurs during hypocapnia overrides the effects due to CO_2 and the vasoconstrictive influence of hypocapnia becomes limited by a vasodilatory hypoxic influence (31). The net result is that a decrease in arterial carbon dioxide tensions to less than 20 mm Hg results in no change or a slight increase in CBF. This hypoxic vasodilation and increase in CBF appears to be related to an increase in anaerobic glucose metabolism with subsequent development of intra- and extracellular tissue acidosis (32). This interpretation is supported by the observation that administration of O_2 allows further decrease in CBF with extreme hypocapnia (33).

Under normal physiological conditions, the influence of oxygen on CBF may be considered negligible. Since the oxygen dissociation curve is relatively flat over a wide range, changes in oxygen tensions over this range do not affect cerebral oxygen delivery and therefore provide little stimulus for CBF change. On the other hand, with increasing degrees of tissue hypoxia the effect of oxygen on CBF becomes increasingly important and predominates over CO_2.

C. Adenosine

The importance of adenosine as one of the factors involved in the metabolic control of the cerebral circulation is currently under investigation. Adenosine has been shown to dilate cat pial arterioles when topically applied (34,35). Furthermore, its concentration rises in the ischemic dog brain (34) and in other conditions where the cerebral

oxygen supply is reduced (36). Consequently, Rubio et al (36) suggested that since brain adenosine and H+ ions were altered in parallel in these conditions they might act together, perhaps synergistically, to regulate the cerebral circulation.

The conditions which increase adenosine concentration produce concomitant changes of H^+ and K^+ in the perivascular space. This raises the possibility of interactions between these ions and adenosine. Moreover further investigation is required to determine the quantitative effects of adenosine on cerebral vessels during altered functional states.

III. NEUROGENIC FACTORS

The recent application of fluorescence and electron microscopy in the investigation of cerebral vessel innervation has confirmed, beyond doubt, that the cerebral arteries possess a rich adrenergic and cholinergic supply (37). These findings have stimulated extensive research effects to identify a functional role for these nerves. Although some studies have provided indirect evidence to support the view that autonomic nerves can influence CBF, the functional significance of cerebral vessel innervation has yet to be established. In the following an overview of recently obtained evidence for and against a functional innervation of the cerebral vessels is presented.

A. Sympathetic System

It is now well established that the adrenergic innervation of cerebral pial arteries and arterioles arises from the superior cervical sympathetic ganglia (38). Superficial cerebral arterioles with a diameter between 100 to 300 microns receive the most dense innervation per vessel size while pial arterioles (diameter 15-20 microns) are less densely

innervated; sometimes accompanied by only one nerve fiber. This pattern of innervation suggests that the larger arterioles would be most strongly influenced by sympathetic activity. It has been suggested however, that since fewer muscle cells are present in the smaller arterioles the presence of only a few nerve fibers could still constitute significant innervation for these vessels (37).

Using a micro application technique to examine the in-vivo cat pial arteries Wahl et al (39) found a significant vasoconstrictor response to norepinephrine in pial arteries ranging in diameter from 30 to 22 microns. The magnitude of the response was independent of vessel size. However, when the mock cerebrospinal fluid in which the norepinephrine was dissolved contained 22 mEq/litre of bicarbonate, norepinephrine had no effect. Maximal response to norepinephrine was observed with a bicarbonate concentration of 11 mEq/litre; a concentration which itself had no effect on vessel diameter. The significance of these results is unclear since normal cerebrospinal fluid bicarbonate concentration in primates is 25 mEq/litre (40). Raper et al (41), using preparations of norepinephrine in artificial CSF containing 25 mEq/litre of biocarbonate, found no response of cat, dog or rabbit pial arteries to direct application of these solutions. Moreover, pial arteries showed no change in vessel caliber with stimulation of the ipsilateral superior cervical ganglion.

Numerous other studies, utilizing a wide variety of experimental approaches, have also been unable to demonstrate significant influence of sympathetic activity on the cerebral arteries. In animals, and in man, α-adrenergic blockade with phenoxybenzamine or phentolamine by intravascular infusion (42,43) or by direct microapplication (44) to pial vessels has no effect on resting CBF or vessel diameter. Similarly, β-

adrenergic receptor blockade with propanolol by internal carotid artery infusion (45) or by director microapplication (46) does not affect the resistance vessels of the cerebral circulation.

The effects of circulating adrenergic amines have been examined in dogs (47), baboons (43), and man (48,49). From these studies it may be concluded that variations in blood catecholamine levels (norepinephrine and epinephrine) have no effect on the cerebral circulation. However, MacKenzie et al (50) have recently reported that cerebral circulatory responses to norepinephrine are dependent upon the integrity of the blood brain barrier. Thus, arterial infusions of adrenergic amines or their blocking agents, may fail to produce adequate concentrations of these agents at the smooth muscle receptor site. This criticism, however, cannot be applied to results obtained using direct perivascular application of these agents.

Recent animal studies in which cervical sympathetic stimulation (electrical) has been performed (51,52) have not demonstrated a significant decrease in CBF under otherwise normal physiological conditions. A similar finding by Skinhøj (42) must be considered important in this regard since this study was carried out in man and sympathetic stimulation was achieved in a physiological manner i.e. distension of the bladder. This stimulus did not alter CBF in the seven patients tested in spite of the marked concurrent increase in blood pressure.

The bulk of available evidence thus indicates that, in the absence of changes in blood pressure, arterial CO_2, or other factors affecting CBF, changes in sympathetic activity exert little or no influence on the cerebral circulation. Further attempts to define a functional role for the sympathetic system in the regulation of cerebral blood flow have been

concerned with the influence of sympathetic activity on CBF responses during altered physiological conditions.

Fraser et al (53) observed a reversal of hypocapneic vasoconstriction in exposed monkey basilar arteries following topical application of the α-adrenergic blocking agent phenoxybenzamine. This finding is in accord with results obtained in cats by Edvinsson et al (54). In this latter study pial artery constriction induced by hyperventilation was released immediately following sympathectomy. James et al (55) found that cerebral blood flow response to changes in $PaCO_2$ in baboons was accentuated following sympathectomy and attenuated during sympathetic stimulation. Conversely, Harper et al (56) demonstrated a significant decrease in CBF during sympathetic stimulation in hypercapneic baboons. Furthermore, in this latter study, intracarotid infusions of norepinephrine at normocapnia had no effect, but resulted in significant decreases in CBF during hypercapnia.

In the light of these observations and the fact that sympathetic fibers innervate the extraparenchymal resistance vessels, Harper et al (56) suggested the presence of a dual control mechanism wherein the cerebral circulation acts as two resistances in series: (i) the extraparenchymal vessels which are influenced by autonomic activity; (ii) the intraparenchymal vessels which are regulated locally by changes in blood gases and cellular metabolism. Thus, under normal conditions changes in the extraparenchymal vessels due to altered autonomic activity can be compensated for by appropriate responses of the intraparenchymal vessels. The summation of these events would result in a constant blood flow. However, if the intraparenchymal vessels are unable to compensate because of dilation due to increased CO_2 or low blood pressure, the effect of

sympathetic activity on cerebral blood flow is now revealed.

Not all observations, however, support such a hypothesis. Intracarotid infusion of norepinephrine during breathing of 8% CO_2, a stimulus which would be expected to maximally dilate intraparenchymal vessels, has no effect on cerebral blood flow in man (57). Waltz et al (58) found no consistent side-to-side differences in cortical blood flow and arteriolar caliber in cats following chronic unilateral superior cervical ganglionectomy. Furthermore, measurements of cerebral blood flow in man during hypercapnia (42) and in baboons during hypocapnia (59) have failed to reveal any significant effect of intravascular infusion of alpha-adrenergic blocking agents under these conditions. As previously noted however, intravascular administration of sympathetic amines or their blocking agents may be ineffective due to barrier mechanisms.

The studies outlined above demonstrate, despite considerable effort, that the physiological role of the sympathetic innervation of the arterial system sypplying the brain is not yet clear. Studies on the effect of neurogenic activity on cerebral circulatory responses to changes in perfusion pressure have also provided conflicting evidence. These studies are discussed in the section dealing with cerebral autoregulation.

B. Parasympathetic System

Studies of cerebral vessels using histochemical techniques have revealed the presence of a well developed cholinergic innervation of cerebral arteries (37). This cholinergic nerve supply occupies an anatomical arrangement on the vessel wall which is very similar to the adrenergic system and, like the adrenergic fibers, extends to arterioles with diameters of 15 microns. In-vivo micro-application of the parasympathomimetic agent carbachol to cat pial vessels has been shown

to induce a vasodilation which can be blocked with atropine (60) suggesting the existence of cholinergic dilatory receptors. Application of atropine in the absence of carbachol, however, has no effect. Therefore, it appears unlikely that resting pial arterial tone is influenced by cholinergic activity.

Parasympathetic cholinergic fibers appear to pass primarily through the facial nerve (61) and there is experimental evidence to suggest that stimulation of the sectioned end of this nerve results in vasodilation and increased cerebral blood flow (62). In contrast, acetylcholine has been demonstrated to cause contraction of the isolated cat middle cerebral artery (63). The reason for these discrepant results is not clear but may be related to the markedly different conditions under which the experiments were conducted.

A study has been recently reported by Hoff et al (64) in which cerebrovascular responses to hypercapnia and hypoxia were tested in baboons following unilateral and bilateral facial nerve section. The response of CBF to hypoxia and hypercapnia was unaffected by facial nerve section and the authors concluded that parasympathetic fibers in the facial nerve play no role in cerebral blood flow autoregulation. On the other hand, intraperitoneal administration of atropine to rats (65) appears to completely block the increase in cortical blood flow associated with a step increase in $PaCO_2$. Thus, while parasympathetic activity may influence cerebral blood flow regulation, the origin and significance of this activity has yet to be determined.

C. Brain Stem Centres

Recent evidence has accumulated suggesting the presence of brain stem centres which participate in the control of CBF. Electrical (66)

and pharmacological (67) stimulation of brain stem structures have been shown to induce large increases in CBF which in large portion are independent of concurrent increases in cerebral metabolism. Shalit et al (26) and more recently Fenske et al (68) have demonstrated abolished responses of the cerebral circulation to increases in $PaCO_2$ following brain stem lesions in animals. Further support for the presence of a central neurogenic influence on cerebral blood flow may be adduced from recent histochemical studies (69,70). These studies have convincingly demonstrated the existence of a 'central noradrenergic system' arising from brain stem centres, (primarily the locus coeruleus) which provides noradrenergic nerve terminals to small intraparenchymal cerebral arteries. The only study (71) reported to date on the function of these nerves has given indication of their importance. In this study the local application of carbachol into the locus coeruleus area in sympathectomized monkeys produced a reduction in CBF and an increase in capillary permeability. The intraventricular administration of the alpha blocker phentolamine had the opposite effect. On the basis of these findings the suggestion was made that the central noradrenergic system is important in the regulation of CBF and capillary permeability.

IV. CEREBRAL PERFUSION PRESSURE - AUTOREGULATION

A. Introduction

Moderate variations in cerebral perfusion pressure, whether induced by changes in blood pressure or intracranial pressure, are normally compensated for by appropriate changes in cerebrovascular resistance so that cerebral blood flow remains constant. The term 'autoregeulation' is now commonly used to described this process.

Attention was first directed to this phenomenon by the studies of

Wolff (72) and Fog (73), who observed that pial arteries dilated in response to a decrease in blood pressure and constricted in response to an increase in blood pressure. More recently, application of quantitative techniques for measuring CBF has repeatedly confirmed the presence of CBF autoregulation in animals and man (4). In pathological studies, varying degrees of autoregulatory impairment have been observed (74), showing that CBF autoregulation is a graded phenomenon.

Under normal physiological conditions CBF remains constant until MaBP is decreased to approximately 60 mm Hg (75). With further reduction in MaBP flow begins to decrease, presumably because cerebral vasodilator capacity is exhausted. Animal studies (76) show that CBF remains constant with increasing intracranial pressure until CPP is 40-60 mm Hg. These observations, and the fact that cerebral vessels dilate with increasing intracranial pressure, indicate that a similar regulatory mechanism exists for both changes in intra- and extravascular pressure. The upper limit of the autoregulatory response to increased MaBP has been investigated in baboons (77), and man (78). In these studies blood pressure levels in excess of 140-150% of the resting value were accompanied by increases in CBF. The finding of a post-hypertension hyperemia in baboons suggests that unphysiological distension of arteries occurs with excessive intravascular pressure thus allowing CBF to increase.

B. Factors Affecting Autoregulation

In CBF experiments involving changes in perfusion pressure, several factors have been shown to significantly alter the normal autoregulatory response. At high $PaCO_2$ levels (greater than 60-70 mm Hg), CBF passively changes in response to a decrease in perfusion pressure. Under these conditions cerebral autoregulation appears to be abolished, presumably

because of the additive effect of two vasodilator factors. On the other hand, the vasoconstrictor stimulus of increased perfusion pressure will maintain or decrease CBF in the presence of cerebral vasodilation induced by moderate hypercapnia (79) or papverine (80). Thus, the blood carbon dioxide level is a major factor determining the level of perfusion pressure at which CBF autoregulation can be demonstrated. The mechanisms which are effective in cerebral autoregulation and CO_2 reactivity, however, are different. Numerous investigations (81,82) have unequivocally demonstrated intact autoregulation in the presence of impaired CO_2 reactivity and vice versa.

Various types of pathological insult to the brain result in impaired CBF autoregulation; induced periods of ischemia (83) and hypoxia (84,85) have been shown to have this effect. It is generally accepted that the mechanism responsible for impaired autoregulation following ischemia or hypoxia is the development of tissue lactic acidosis which causes 'paralysis' of cerebral arteries.

C. Mechanism of Autoregulation

Studies of CBF autoregulation have not answered the fundamental question as to the mechanism by which this response is effected. At present, three theories retain wide support.

(1) The myogenic theory postulates that the stimulus for changes in arterial diameter is a change in transmural pressure (intravascular-extravascular pressure); the smooth muscle of the artery responding to an increase in pressure with contraction and vice versa. A resting tone is implied for normal perfusion pressure.

(2) The metabolic theory for CBF autoregulation implies an initial passive flow change in the direction of change in perfusion pressure.

The flow change then alters the chemical milieu of the tissue, for example, the local concentration of CO_2 (and therefore H+), which secondarily induces changes in arterial diameter to keep flow constant.

(3) The neurogenic theory postulates that neurogenic influences control, or at least modify CBF responses to changes in perfusion pressure.

Much indirect evidence has been presented to support both the myogenic and metabolic theories. However, differences in the methodology employed, a wide variety of experimental designs, and inattention to other factors affecting CBF in these studies have probably contributed greatly to the lack of consensus that still exists. This is exemplified by consideration of two recent studies.

Symon et al (79) studied hemodynamic reactions to rapid pressure changes in an isolated vascular are of the cerebral circulation in the baboon. This was accomplished by catheterization of Labbe's vein and a branch of the middle cerebral artery with monitoring of outflow in the vein. Acute pressure increases in the artery resulted in rapid appropriate adjustment of cerebrovascular resistance, often complete within 10-15 seconds. The authors, arguing that matabolic changes in cerebral vascular resistance could not occur with such a short latency, concluded that their findings were best explained on the basis of the myogenic theory.

In the second study, also in baboons, Kawamura et al (86) tested autoregulation by inducing increases in blood pressure with an inflatable balloon positioned in tha aorta. Cerebral blood flow was measured with electromagnetic flowmeters around both internal jugular veins. Acute increases in perfusion pressure resulted initially in an increase in CBF which gradually returned to steady state levels in 3-5 minutes. Cerebrovascular resistance increased gradually over this same period. This type

of response, in which cerebrovascular resistance slowly adjusts to a change in cerebral perfusion pressure, is thought to be compatible with a metabolic mechanism of autoregulation.

The results of investigations on the role of neurogenic influences in cerebral autoregulation are also inconsistent. CBF responses to alterations in perfusion pressure are normal in animals with chronic sympathectomy (87,88). Some investigators (81) have reported impaired autoregulation in patients which acquired autonomic insufficiency (89) while others (90) have reported negative results. Alpha-adrenergic blockade does not affect CBF responses to a decrease in perfusion pressure (88) but appears to impair autoregulatory constriction to an increase in perfusion pressure (86). Atropine administered into the vertebral or carotid arteries (91) does not affect autoregulation. On the other hand, the anticholinesterase agent neostigmine has been reported to impair the cerebral vasoconstrictor response during intracarotid and intravertebral infusion of this agent (92).

From the studies outlined in the above it is apparent that the mechanisms controlling CBF autoregulation are far from clear. It is equally apparent that autoregulation is a complex phenomenon and is unlikely to be an exclusive property of any one factor or of a particular portion of the cerebral vasculature.

CHAPTER TWO

PATHOPHYSIOLOGY OF THE CEREBRAL CIRCULATION IN SUBARACHNOID HEMORRHAGE

I. INTRODUCTION

Clinical studies (93,94) indicate that the initial response to extravasation of blood from a ruptured cerebral aneurysm is an abrupt rise in intracranial pressure which tends to approach mean blood pressure levels. During the period of peak elevation in intracranial pressure CBF is severely reduced. This reduction in flow allows hemostatic mechanisms such as clotting to take place. Once clotting has occurred spatial compensation in the cerebrospinal fluid compartments permits ICP to decrease to less dangerous levels without further hemorrhage from the aneurysm. Presumably, failure of hemostasis by clotting would result in continued hemorrhaging, high ICP, low CBF and rapid progression to fatal cerebral ischemia.

Some patients surviving the initial effect of a SAH may do so without evidence of brain injury while others demonstrate varying degrees of neurological deficit. However, the subsequent clinical course of these patients is variable and appears to be related to three major factors:

(1) Recurrent hemorrhage from the aneurysm.

(2) Onset of cerebral arterial spasm.

(3) Development of cerebral ischemia.

At present, little is understood of the changes in the cerebral circulation which permits a recurrence of the SAH. That it typically occurs at 7 to 14 days following the initial hemorrhage (94) is suggestive of dissolution of the hemostatic clot. Release of spasm in arteries proximal to the aneurysm and a decrease in ICP have also been

suggested as contributing factors in recurrent SAH.

The incidence of cerebral vasospasm subsequent to aneurysm rupture is approximately 40% (95,96,97). In man angiographically visualized vasospasm is usually delayed in onset by 3 to 4 days and may last up to 3 to 4 weeks (98,99).

Spasm of the cerebral arteries may be localized or diffuse. Local spasm occurs as a preferential narrowing in one or more vessels, usually near the aneurysm, and affects only a short segment of an artery. Diffuse spasm is characterized by a generalized narrowing of the cerebral arteries. Wilkins et al (96) examined the records of 120 patients with spontaneous SAH and suggested that spasm begins as a local event adjacent to the aneurysm and is subsequently propagated to other areas.

II. CEREBRAL VASOSPASM - PATHOGENESIS

Because of widespread belief that cerebral vasospasm is the major factor contributing to the development of cerebral ischemia in patients with SAH, intensive research has been carried out in an attempt to identify the factor(s) in blood which are capable of producing prolonged constriction of the cerebral arteries. Most investigators have concluded that mechanical irritation produces only localized, transient vasospasm.

Among the blood-borne factors which are capable of causing cerebral vasoconstriction, norepinephrine, prostaglandins E_2 and $F_{2\alpha}$ and serotonin have been the most widely investigated. However, only serotonin appears to fulfill the criteria suggested by Zervas (100) as being necessary for defining an agent responsible for cerebral vasospasm:

(1) The substance must be vasoactive.

(2) The substance must be recovered at the site of SAH

(3) A specific antagonist to this agent must prevent spasm.

(4) Removal of this agent must relieve spasm in the experimental model.

Serotonin (5-hydroxytryptamine) is a highly active vasoconstrictor which is widely present in mammalian tissues. However, all the serotonin present in blood can be found in concentrated form in the blood platelets (101). During clotting, the action of thrombin on blood platelets causes release of approximately half the serotonin, the remainder being released slowly over several days. These properties of serotonin led early investigators to postulate that it might be responsible for the cerebral arterial spasm which was being observed in patients with subarachnoid hemorrhage (SAH) from rupture of an intracranial aneurysm.

Early experimental attempts to implicate serotonin in post-SAH cerebral vasospasm produced widely varying results. Although serotonin invariably caused constriction when applied topically to exposed cerebral arteries (102, 103, 104), the degree of constriction observed in one such study was markedly less than that obtained with blood (103). In another study, marked constriction of the basilar artery was produced by an apparently serotonin-free fraction of blood (104). Furthermore, although intracarotid infusion of serotonin in monkeys produced cerebral vasoconstriction (105), no difference in serotonin blood levels were found between patients with SAH (with or without cerebral vasospasm) and a control group (106).

In contrast with the afore-mentioned results, recent investigations have produced substantial evidence indicating that serotonin plays a major role in the genesis of post-SAH cerebral vasospasm. The results of these investigations are summarized below.

(1) Depletion of blood serotonin levels by 50-75% prevented the

occurence of cerebral vasospasm following middle cerebral artery puncture in monkeys (107) or cisterna magna injection of blood in dogs (108).

(2) In-vitro experiments utilizing segments of dog cerebral arteries as a bioassay system demonstrated that:

(a) Only serotonin (out of numerous vasoactive substances tested) was capable of producing maximal cerebral artery contraction at a concentration similar to that found in clotted blood (109).

(b) The contractile activity of cerebrospinal fluid collected from patients with SAH was due to serotonin (110).

(3) Cisterna magna injections of blood or serotonin at physiological concentrations produced quantitatively similar degrees of spasm of the dog basilar artery. Cisternal injection of phenoxybenzamine (a serotonin antagonist), relieved both the serotonin and blood induced spasm (111).

(4) In-vitro bovine middle cerebral artery segments contracted when exposed to concentrations of serotonin found in human plasma and serum (112).

(5) Human cerebrospinal fluid from patients with SAH and cerebral vasospasm caused marked contractions of isolated human cerebral arteries (113).

A causal relationship between serotonin and vasospasm is strongly indicated by the studies mentioned above. However, clinical studies using agents known to block the vasoconstriction effects of serotonin have produced conflicting results (114, 115). Thus, the role of serotonin in the pathogenesis of cerebral vasospasm in man has not yet been defined.

CHAPTER THREE

EXPERIMENTAL STUDIES - MATERIALS AND METHODS

A description of the materials and methods utilized in the experimental studies is presented below. Many of the techniques were unchanged throughout the course of the studies. In some areas changes were made and these are pointed out to the reader. However, unless specified otherwise the techniques described pertain to all experiments.

The first study (Study A) was an investigation of the effect of SAH on CBF autoregulation. In the second study (Study B), the effects of subarachnoid blood and serotonin on the cerebral circulation were examined and compared.

I. ANIMAL PREPARATION

Juvenile and adult female rhesus (macaca mulatta - Study A) and cynmolgous (macaca irus - Study B) monkeys were utilized. Sedation was achieved with intravenous sodium pentothal (25-30 mg/kg). Endotracheal tubes were introduced and the animals were ventilated with a Harvard variable phase respirator. Paralysis of the animals was induced with intravenous tubocurarine (Study A) or gallamine (Study B) and maintained with additional supplements as required. Light general anesthesia was maintained throughout the experimental period with a mixture of nitrous oxide (N_2O) and oxygen (O_2) from a reservoir in a ratio of 2 to 1.

Body temperature was continuously monitored by an esophageal thermometer (Tele-Thermometer-Yellow Springs Instrument Co.) and maintained between 36 and 38 degrees centigrade by a small heating pad positioned below the animals. Standard lead electrocardiography (Beckman Dynograph (type R) Recorder) was performed in all animals.

Femoral artery catheterization was performed in the anesthetized

animals and arterial blood samples were immediately obtained for pH and blood gas analysis (Instrument Laboratory Inc., pH/Gas Analyzer, model 113). By adjusting the volume output from the respirator, arterial pH, carbon dioxide ($PaCO_2$) and oxygen (PaO_2) values were kept within the physiological range during surgery. Thereafter, arterial pH and blood gas analysis was performed during each measurement of cerebral blood flow. Hematocrit determinations, required for CBF calculation, were carried out frequently (4 to 6 times) in the course of each experiment.

Mean arterial blood pressure (MaBP) was continuously monitored by a pressure transducer (Statham $P_{23}dB$) connected to the femoral artery catheter. Cerebrospinal fluid pressure (CSFP) was also continuously monitored by another pressure transducer (Statham $P_{23}dB$) connected to a catheter positioned in the lumbar subarachnoid space. Both pressure transducers were calibrated against a mercury manometer prior to each experiment. Zero drift was checked frequently during the experiment and adjusted as required. In Study A a cannula was positioned in a femoral vein for continuous infusion of the pressor drug aramine bitartrate (40-50 mg in 500 cc normal saline).

A cranial twist-drill hole (1.5 mm diameter), 0.5 to 1.0 cm dorsal to the nasion, was performed in the animals to be subjected to subarachnoid injections. Hemostasis was achieved with bone wax application and the defect was sealed until the time of subarachnoid injection.

Cervical dissection to the common carotid artery bifurcation was performed with the aid of an operating microscope (Codman Mark II). In Study A the external carotid artery was isolated and doubly ligated immediately distal to the origin of the external maxillary artery. The external maxillary artery was carefully dissected from surrounding tissues

and doubly ligated approximately two centimeters distal to its origin. A 21-gauge polyethylene catheter was then inserted into the internal carotid artery via the external maxillary artery. The catheter was connected to an injector device (Appendix I) via a 3-way stopcock.

In Study B the cervical portion of the common carotid artery was carefully exposed. A 7-0 silk purse-string suture was placed in the wall of the common carotid artery approximately two centimeters proximal to the bifurcation. The diameter of the circle formed by the purse-string suture was approximately one millimeter. A 20-gauge catheter (Medicut) was then inserted into the common carotid artery through the space enclosed by the suture and threaded to the origin of the internal carotid artery. The external carotid artery was clipped at its origin using a modified aneurysm clip. The carotid artery catheter was then connected to the injector device via a 16-gauge catheter and 3-way stopcock assembly. At the end of the experiment the carotid catheter was removed and the purse-string suture tightened to close the small arteriotomy.

II. METHOD OF SUBARACHNOID INJECTION

Prior to subarachnoid injection a circumferentially beveled, 19-gauge spinal needle was carefully inserted (under fluoroscopy) along the floor of the anterior fossa into the chiasmatic cistern. Adequate placement of the needle was confirmed with return of cerebrospinal fluid after removal of the needle stylette. The needle was then secured to the skull by a screw device.

In Study A subarachnoid hemorrhage was induced by manual injection of 4 ml of fresh autogenous arterial blood under constant pressure. Duration of the injection was approximately 25 seconds. Mock subarachnoid hemorrhage (MSAH) was induced by the same procedure using artificial

cerebrospinal fluid (113) at normal pH (≈7.35) and temperature (37°C).

In Study B 3 ml of fresh autogenous arterial blood, artificial cerebrospinal fluid or serotonin solution were injected into the subarachnoid space using the same procedure as in Study A. In all experiments artificial cerebrospinal fluid was freshly prepared from stock solutions just prior to subarachnoid hemorrhage. The serotonin solution was prepared by mixing a small volume of serotonin stock solution (0.5 ml at 1×10^{-3} M) into 100 ml of artificial cerebrospinal fluid to produce a final serotonin concentration of 5×10^{-6} M.

III. ADMINISTRATION AND DETECTION OF ^{133}Xe.

Cerebral blood flow was measured by the ^{133}Xenon intra-arterial injection method development by Lassen, Ingvar and their associates (117).

An automatic injector device was utilized to inject 2.5 to 3.5 millicuries of ^{133}Xe into the internal carotid artery. The construction, operating characteristics, and in-vivo testing of the injector system are described in detail in Appendix I.

Regional clearance rates for ^{133}Xe were measured by a six-detector scintillation counter assembly constructed in this laboratory (Fig. 1). Four of the detectors recorded the radioactivity from supratentorial regions - frontal, parietal, occipital and temporal (detector # 1, 2, 3 and 5 respectively). Detector #4 and #6 recorded radioactivity from the orbito-maxillary and cerebellar regions respectively.

Each scintillation counter comprised of 0.60 centimeters diameter, 1.25 centimeter thick sodium-iodide-thallium activated crystal coupled to a 1.25 centimeter diameter photomultiplier (Phillips XP 101) with a truncated plexiglass light guide. The detectors, spaced at a distance of 1 centimeter from each other, were mounted in a stainless steel collimator

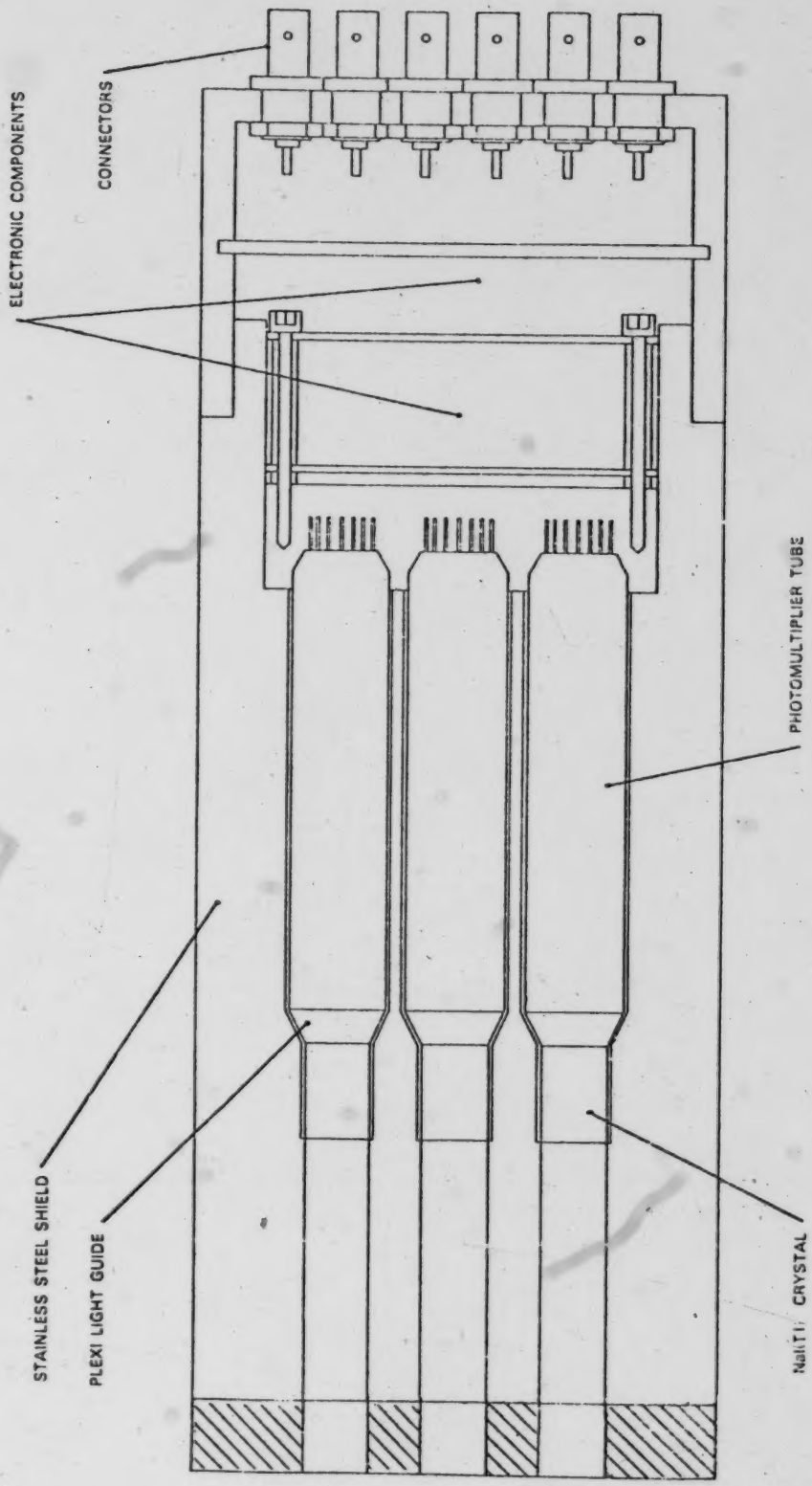


Fig.1 Multidetector System (Superior view)

block with the front face of each crystal recessed 7.5 centimeters from the block face. An additional 1.5 centimeter lead collimator applied to the block face ensured measurement of radioactivity from discrete volumes of brain tissue. The isoresponse curves for two adjacent collimated detectors are shown in Figure 2. Radioactivity was also measured from the contralateral side using a large scintillation counter with a 2.54 centimeter diameter 1.27 centimeter thick sodium-iodide-thallium activated crystal. However, only the regional cerebral blood flow data was utilized in the data analyses.

Pulses from each detector were amplified and then input to single channel analyzers which accepted pulses with energy greater than 70 Kev (Fig. 3). The output (sampling time-2 seconds) from each single channel analyzer was fed into the data acquisition system. Visual display of the ^{133}Xe clearance from each brain region was provided by a small monitor located within the laboratory. Data acquisition and retrieval was accomplished via a control teletype also located in the laboratory. The radioisotope clearance curves were analyzed by an on-site digital computer. Background radiation was recorded for a two-minute period and a calculated mean value was subtracted from the clearance curves prior to computer calculation of regional cerebral blood flow values.

Correct placement of the multidetector system was achieved by applying a plastic template (with six radiopaque circular markers with diameters identical to the detectors) to the lateral surface of the skull. Manipulation of the monkey's head or adjustment in the position of the detector assembly under fluoroscopy ensured accurate positioning of the multidetector system (Fig. 4).

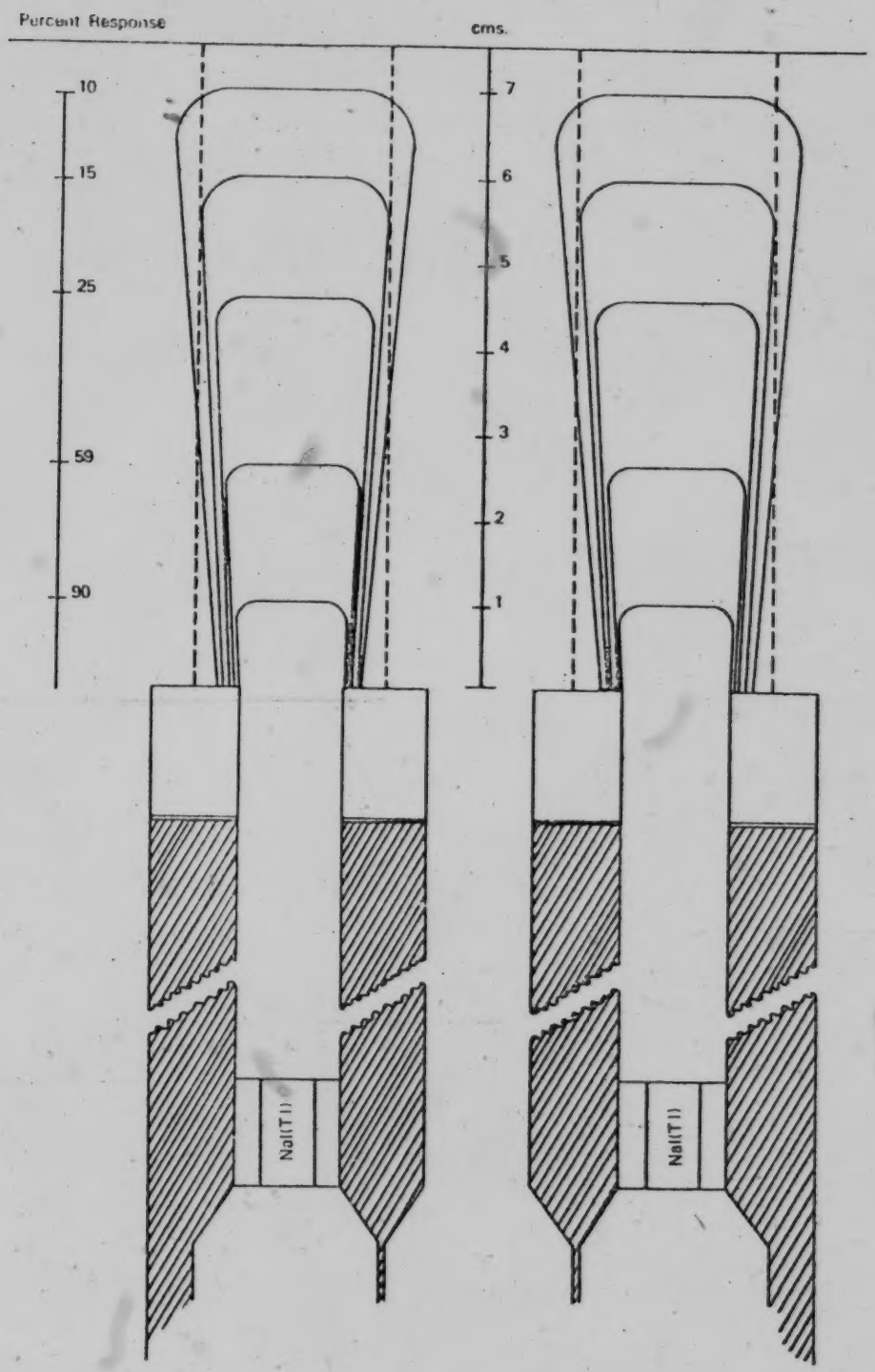


Fig. 2 Isoresponse curves for two adjacent collimated detectors with a point course of 133 xenon in water. Isocount contour at the 90, 50, 25, 15 and 10 percent response levels.

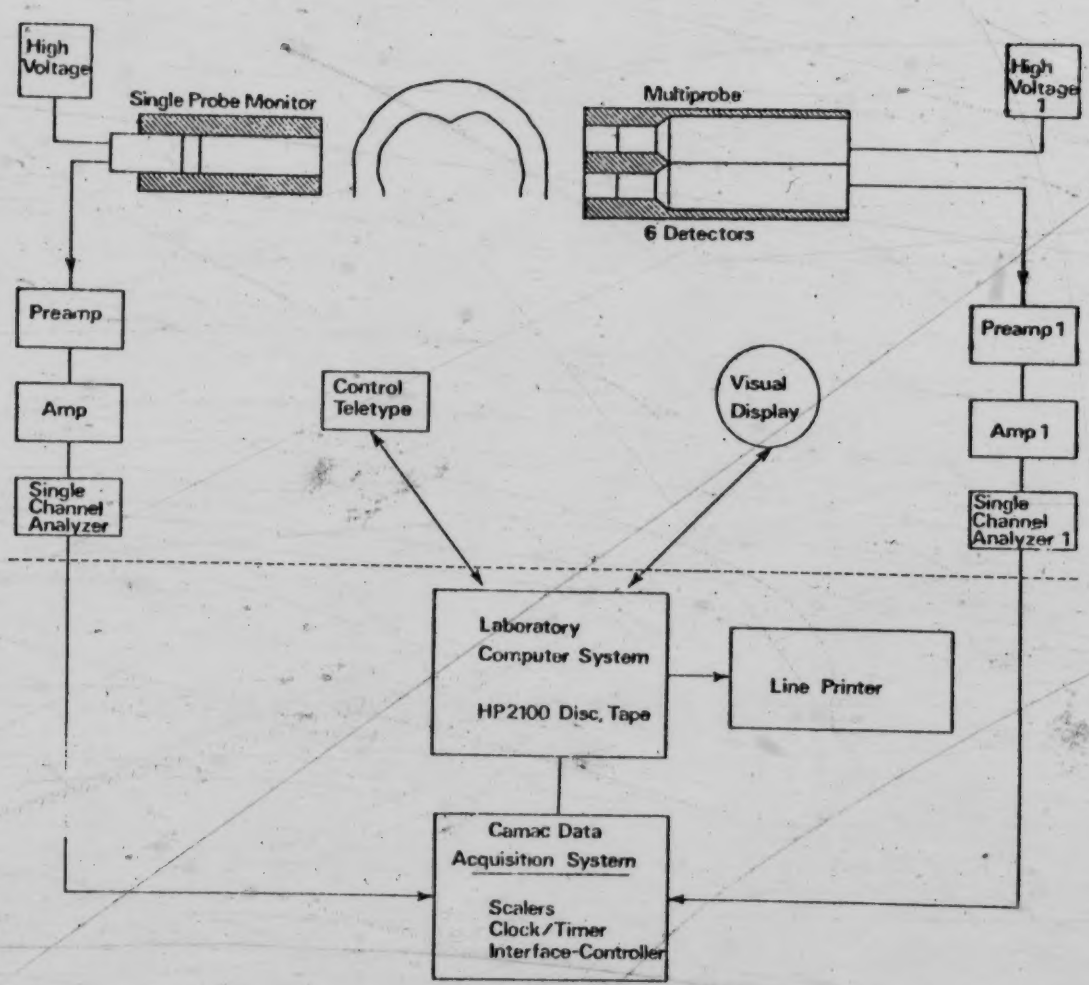


Fig. 3 Instrumentation for partial hemispheric and regional cerebral blood flow studies.

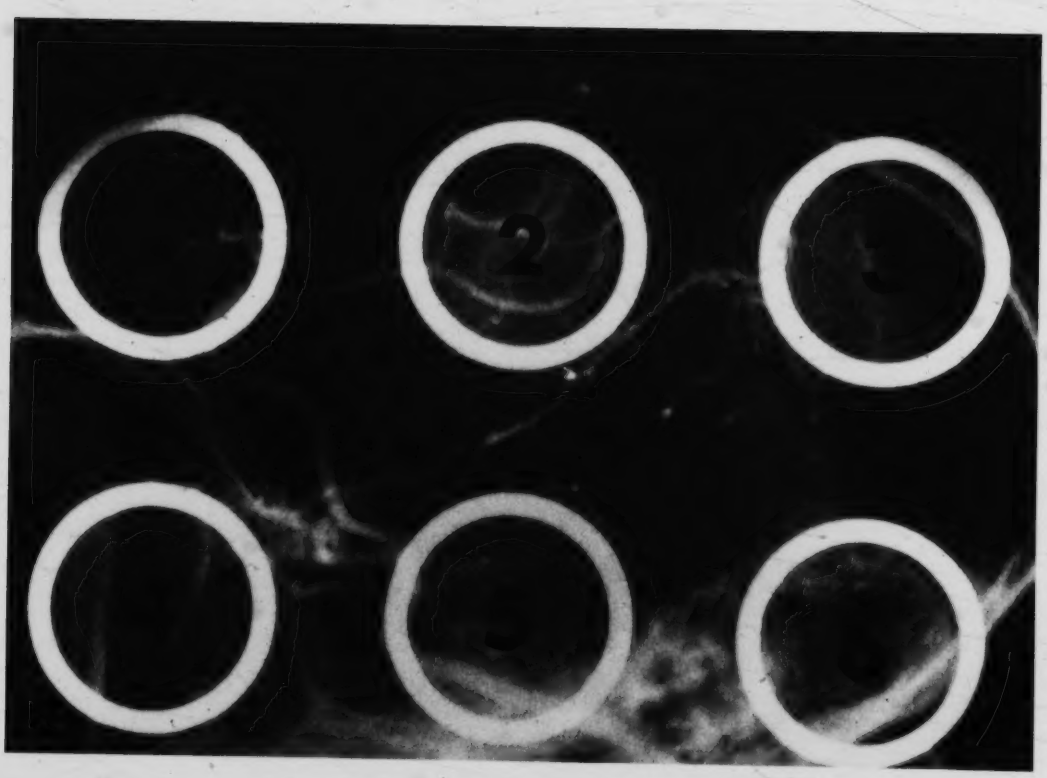


Fig. 4 Lateral cerebral angiogram showing scintillation detector placement. Detectors 1, 2, 3 and 5 measure rCBF from the frontal, parietal, occipital and temporal areas of the brain. Cerebellar and orbitomaxillary perfusion is measured by detectors 6 and 4 respectively.

Mean hemispheric blood flow (mHBF) was determined by averaging flow values from the four supratentorially placed detectors (probes 1, 2, 3 and 5).

IV. CALCULATION OF CEREBRAL BLOOD FLOW

Cerebral blood flow values utilized in the data analysis were calculated by the height/area (H/A) and initial-slope-index methods (ISI). Many detailed discussions of these methods of CBF calculations have been presented (117,118) and will only be reviewed here.

The H/A method formulated mathematically by Zierler (119) can be applied to calculate the mean flow values from the clearance curve according to the equation:

$$rCBF = \frac{(H_0 - H_{10})}{A_{10}\text{-Background}} \cdot \lambda \cdot 100 \text{ ml/100gm/min}$$

where H_0 corresponds to the total number of indicator particles to be cleared, and H_{10} the level of the clearance curve at 10 minutes. A_{10} is determined by integrating the counts recorded during 10 minutes and λ denotes the mean tissue: blood solubility coefficient for ^{133}Xenon.

The initial-slope-index (ISI) method utilizes the calculated slope of the first 1.5 to 2.0 minutes of the logarithmic clearance curve. An estimate of the mean CBF can be readily determined using:

$$rCBF_{ISI} = 100\lambda_g \cdot 2.30\ D_{initial} \text{ ml/100gm/min}$$

where λ_g corresponds to the ^{133}Xenon partition coefficient of grey matter and D represents the numerical value of the slope of the curve.

Because of the profound influence of carbon dioxide on cerebral

perfusion, particular care was taken to maintain $PaCO_2$ levels within the physiological range during steady state studies. Both uncorrected and corrected flows were calculated; corrected (to $PaCO_2$ = 40 mm Hg) flow values were utilized in subsequent data analysis. For each millimeter change in $PaCO_2$ a correction factor of 2.5 percent for CBF change in the same direction was incorporated into the computer program (120). In the post-hemorrhage period special care was taken to keep $PaCO_2$ values near 40 mm Hg because of a possible effect of the subarachnoid injections on carbon dioxide responsiveness.

The partition coefficient for ^{133}Xe in the grey and white matter was calculated according to the method of Veall and Mallett (121) using the solubilities of ^{133}Xe in the grey and white matter of baboons. Since the hematocrit influences the solubility coefficient of ^{133}Xe in whole blood and the blood-brain partition coefficient, a correction equation as described by Veall and Mallett (121) was incorporated into CBF calculation. A mean partition coefficient for the brain was calculated on the basis of a 52:48 grey to white matter ratio as determined for the baboon brain by James (122).

The calculation of CBF presupposes that the clearance curve represents a single transit of the tracer and that the tracer does not recirculate to the counting field. Even though the lungs function as an effective filter for inert gases with low solubility (90 percent of ^{133}Xe is cleared during the first passage through the lungs and the remaining 10 percent is distributed in the total cardiac output with only 14 percent of the recirculating activity distributed to the brain) recirculation does occur. However, only in the presence of gross pulmonary insufficiency or with a rebreathing ventilation system does recirculation become a

significant factor in CBF determination (117). In the present experiments exhaled ^{133}Xe was removed by a hose at a slight negative pressure to the respirator. Correction for recirculation was not performed.

V. ANGIOGRAPHICAL STUDIES

Cerebral angiographical studies were performed in all animals by forceful injection of 1.0 to 1.5 millilitres of meglumine iothalamate (Conray 60) into the internal carotid artery. Only lateral angiograms were obtained and care was taken to maintain magnification factors constant during each experiment.

Only the large intradural vessels were measured; intradural internal carotid (IDICA), middle cerebral (MCA), proximal pericallosal (PPA), and distal pericallosal (DPA) (Fig. 5). Arterial (intraluminal) calibers at these perdetermined fixed locations were measured with a micrometer lens system positioned at a fixed focal distance from the film. Of the two to three films obtained from each angiographical sitting, the one showing the arterial phase most distinctly was selected for measurement studies. Each artery was measured four times and the mean values thus obtained were utilized for statistical analysis. Comparison of arterial calibers in 30 pairs of films revealed no significant difference between measurements obtained from the first or second film (taken two to three minutes apart).

VI. NEUROLOGICAL ASSESSMENT

Neurological examination was performed in all monkeys in the post-anesthetic period. A five division neurological grading system was utilized for evaluation of the animals.

Grade I: alert, active and vocal, no evidence of neurological deficit, accept food and water.

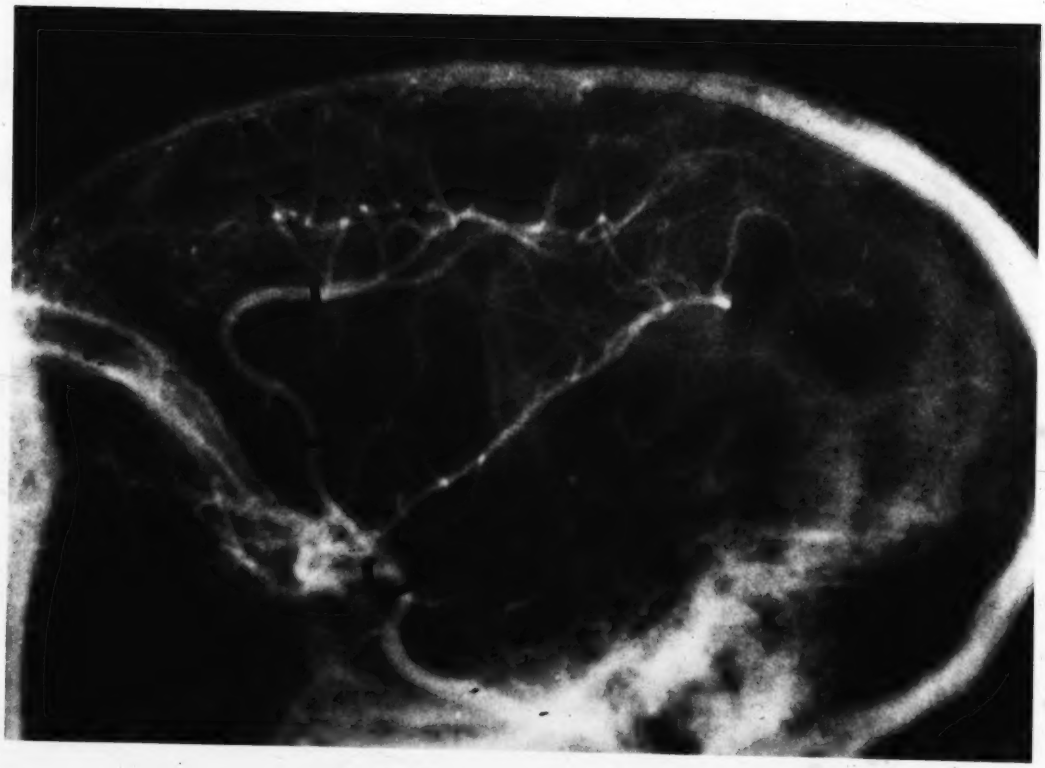


Fig. 5 Lateral cerebral angiogram showing sites of cerebral arterial caliber measurements.

Grade II: mildly obtunded, not as active or vocal, no significant neurological deficit.

Grade III: moderately obtunded, neurological deficit (i.e., hemiparesis, paraparesis, cranial nerve palsy), usually assume a semi-supine position but sit up when stimulated, respond to all forms of stimulation (auditory, touch, pain).

Grade IV: Severely obtunded, severe neurological deficit (i.e. hemiplegia, quadraplegia), little or no response to painful stimulation, frequently exhibit generalized intermittent clonic seizures of variable duration.

Grade V: moribund, unresponsive to all forms of stimulation, failing vital signs (falling BP, arrhythmias, shallow irregular respirations).

VII. PHARMACOLOGICAL IN-VITRO STUDIES

The tissues utilized in the in-vitro studies were:

(1) Human umbilical cord arteries obtained within 30-45 minutes following delivery.

(2) Dog and monkey cerebral vessels removed within one-half hour of sacrifice by exsanguination.

(3) Rat stomach fundus strips obtained within 10-15 minutes of sacrifice.

Tissues were placed into cooled oxygenated modified Krebs-Henseliet solution after dissection. Spiral strips were then cut and mounted in jacketed 5 or 10 ml organ baths maintained at 37 degrees centigrade and aerated with a 5% CO_2/95% O_2 mixture (pH = 7.4). After a 2 hour recovery period the response of the tissue to various agents (serotonin, cyproheptadine, monkey serum) was tested. Isometric tissue responses were measured using a force displacement transducer (Grass-Model FT) connected to a Grass 4-channel polygraph (model 5-D) or a Beck dynograph (Model B). The modified Krebs-Henseliet solution bathing the tissues in the organ baths had the following composition (mM): NaCl 116;

KCl, 5.4: $CaCl_2$, 2.5; NaH_2PO_4, 1.2; $MgCl_2$, 1.2; $NaHCO_3$, 22.0; D-glucose, 11.2.

Monkey serum serotonin concentrations were estimated by comparing serum-induced contractions to contractions obtained using known concentrations of serotonin. A minimum of two concentrations of serotonin and two concentrations of serum were utilized for each serum serotonin determination (4-point assay).

In other in-vitro experiments, the response of the tissue (monkey cerebral arteries) to cumulative concentrations of serotonin or specific concentrations (5×10^{6} M) of serotonin and serum were determined in the presence and absence of the serotonin antagonist cyproheptadine at a concentration of 5×10^{-6} M.

VIII. ELECTRON MICROSCOPY STUDIES

Fixation of monkey cerebral vessels was performed by intracardial infusion with Krebs-Ringer solution containing 4 percent glutaraldehyde in Millonig's phosphate buffer. After perfusion for approximately 10 minutes (500-600 cc) the brain was carefully removed. Samples of vessels from the left cerebral circulation were removed at sites which corresponded to the sites used for angiographic determinations of vessel caliber - IDICA, MCA, PPA and DPA. A small (3mm x 3mm x 2mm deep) block of cerebral cortex and leptomeninges was removed from the superior portion of the precentral gyrus and from the superior temporal gyrus. All samples were placed in small vials containing the perfusion fixative immediately upon removal from the brain.

Tissues were postfixed in 1 percent osmium tetroxide, dehydrated in a series of ethanols, and imbedded in Epon. After staining, sections were viewed with a JEM-7A electron microscope.

CHAPTER FOUR

EXPERIMENTAL STUDIES-OBJECTIVES

EXPERIMENTAL DESIGNS, RESULTS AND DISCUSSIONS

Two major experimental studies were conducted. Each study examined different but related pathophysiological aspects of SAH. There are components which are common to the two studies, however, for the purpose of clarity, each is presented below as a separate investigation. Both studies are presented in a similar fashion.

I. STUDY A - THE EFFECT OF SAH ON rCBF AUTOREGULATION AND CEREBRAL VASOMOTOR TONE

A. Objectives

(a) Determination of normal rCBF autoregulatory response to increases in cerebral perfusion pressure.

(b) To examine the effect of SAH on rCBF autoregulatory response and vessel caliber response to increases in cerebral perfusion pressure and to compare these effects to those obtained with a MSAH.

(c) Determination of normal vessel caliber responses in the major cerebral arteries to increases in cerebral pressure.

(d) To document the neurological status of the animals following SAH and MSAH.

B. Experimental Design

Regional CBF response to increases in MaBP was determined in 17 rhesus monkeys during a pre-hemorrhage control period (3-5 hrs.). Prior to beginning rCBF measurements, baseline angiograms were obtained and examined for evidence of surgically induced trauma to the cerebral arterial system. Two to four rCBF flow measurements were then carried out at resting MaBP levels, following which MaBP was increased in steps of 10-15 mm Hg and a rCBF measurement was done at each MaBP level. MaBP increases were induced using intravenous infusions of metaraminol bitartrate (Aramine). A minimum of 5 minutes was allowed for stabilization of the cerebrovascular response to an increase in MaBP before carrying out a flow measurement. In general, the maximum increase in MaBP was 30-40% above the resting value.

Following completion of pre-hemorrhage testing of rCBF autoregulation, angiography was performed at resting MaBP levels, and within 10-15 minutes at hypertensive levels.

SAH was induced in 12 animals. In a control group, comprising 5 animals, MSAH was induced. Hemorrhage was always induced during normotension. After allowing 15-20 minutes for stabilization of the effects of a hemorrhage, rCBF autoregulation to increases in MaBP was again tested in the same manner as the pre-hemorrhage test of autoregulation. Upon completion of post-hemorrhage testing of autoregulation, angiography was again performed at resting MaBP levels, and within 10-15 minutes at hypertensive levels.

The animals were then allowed to recover from the anesthesia. They were observed for 1-2 hrs and their neurological status was evaluated. They were then sacrificed and the brains removed for gross pathological examination.

C. Results

(a) Cardiovascular and cerebrospinal fluid pressure responses.

The values of $PaCO_2$, pH and CSFP obtained during the rCBF studies are summarized in Table 1. Care was taken to maintain $PaCO_2$ near 40 mm Hg during rCBF measurements and angiography. CSFP was measured in 6 animals with SAH and in 3 animals with MSAH. Mean CSFP was significantly elevated ($p<0.01$) during the post-SAH period but remained at normal levels during the period following MSAH. The significant elevation in CSFP observed during the post-SAH period appeared to be partly due to moderate increases in CSFP which occurred when MaBP was increased. Therefore, a linear regression analysis of the relationship between CSFP and MaBP was performed (Fig. 6). The regression equation is: CSFP = 0.73 + 0.13 MaBP. The slope of the regression line is significantly different ($p<0.01$) from zero.

During subarachnoid injection of blood or artificial CSF, CSFP increased rapidly; the peak pressure occurring 20-30 seconds after the beginning of the injection. A typical example of cardiovascular and CSFP responses to a 4cc injection of blood is shown in Fig. 7. When CSFP approached MaBP levels the MaBP began to increase (Cushing response), and in some animals was associated with transient (2-3 min) bradycardia and EKG changes. Sinus arrythmias, premature ventricular contractions, increased T-wave amplitudes and S-T elevations were noted. The mean CSFP and standard deviation occurring with injection of 4 ml of blood and artificial CSF was 141±58 mm Hg (n=6) and 139±9 mm Hg (n=3) respectively. CSFP and MaBP levels stabilized within 3-5 minutes following subarachnoid injection.

TABLE 1

GROUP	SAH		MSAH	
	PRE-SAH	POST-SAH	PRE-MSAH	POST-MSAH
$PaCO_2$	40.3 ± 2.9	37.9 ± 5.9	40.7 ± 3.8	38.0 ± 4.0
PaO_2	123.2 ± 8.6	130.6 ± 8.0	121.6 ± 9.6	122.4 ± 6.7
pH	7.41 ± 0.04	7.39 ± 0.05	7.38 ± 0.08	7.39 ± 0.09
CSFP	7.9 ± 3.7	18.4 ± 5.1	8.7 ± 1.9	8.6 ± 1.5

SAH- subarachnoid hemorrhage

MSAH- mock subarachnoid hemorrhage

Mean and standard deviations of blood gases, pH and cerebrospinal fluid pressure (CSFP) in animals subjected to SAH and MSAH.

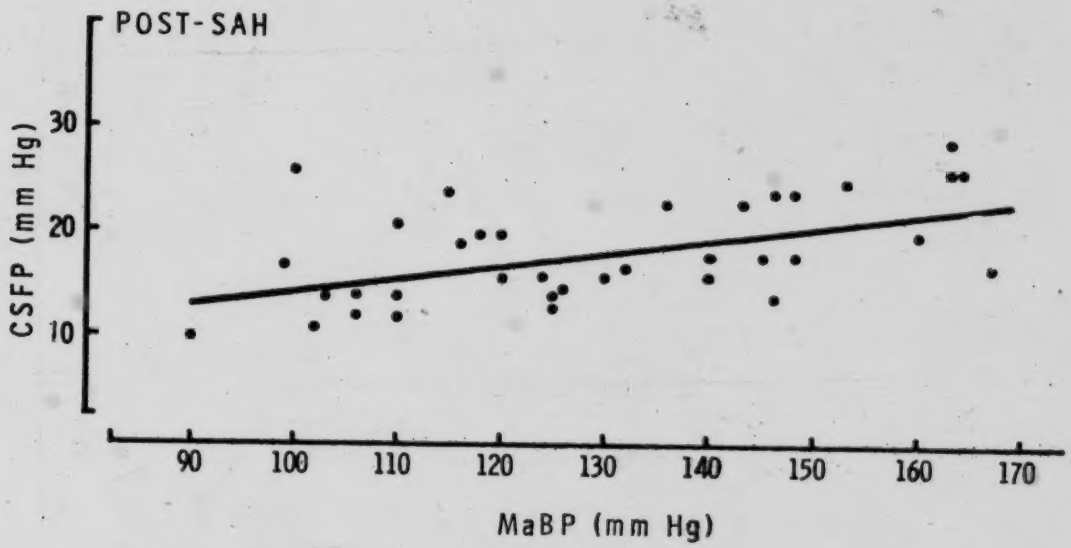


Fig. 6 Post-SAH relationship between cerebrospinal fluid pressure and MaBP.

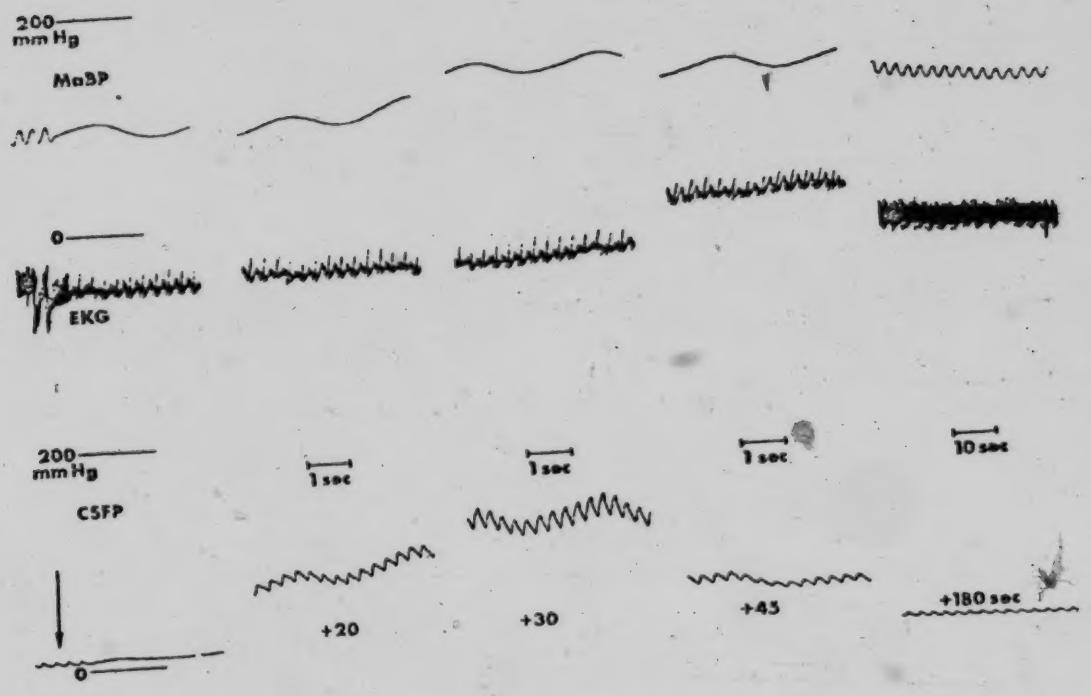


Fig.7 Cardiovascular and cerebrospinal fluid pressure (CSFP) responses to subarachnoid injection of 4 ml. of blood. Arrow indicates start of injection.

(b) Autoregulation

Pre- and post-SAH tests of autoregulation were conducted in 17 monkeys. CBF (mean hemispheric blood flow) response to increases in MaBP in one animal from the SAH group is illustrated in Fig. 8. In this animal, pre-SAH CBF remained relatively constant despite large increases in MaBP. Following SAH, CBF increased passively with increases in MaBP. Regional CBF autoregulation in this same animal is shown in Fig. 9 A B C D. Before SAH, an increase in MaBP from 121 to 151 mm Hg had no effect on CBF in any of the 6 regions measured. When autoregulation was again tested during the 3 hour period following SAH, an increase in MaBP from 120 to 150 mm Hg caused a marked increase in CBF in all regions.

Linear regression analysis of the pre- and post-hemorrhage group data was carried out (Fig. 10). The slope of the regression line for the pre-hemorrhage data is positive, but not significantly different ($p>0.05$) from zero. This regression line was used as an index of intact autoregulation and compared with the results obtained from analysis of the post-hemorrhage data. The results show that the slope of the post-MSAH regression line was not significantly different from the slope of the pre-hemorrhage line. In contrast, the post-SAH regression line slope was significantly greater ($p<0.01$) than the slope of the pre-hemorrhage line.

(c) Cerebral Vessel Reactivity

A linear regression analysis of the relationship between angiographically determined cerebral vessel caliber and MaBP was performed (Fig. 11). Data obtained from measurements of the intradural internal carotid artery (IDICA) was used for the analysis. Since the IDICA was the vessel most consistently well visualized on lateral angiograms,

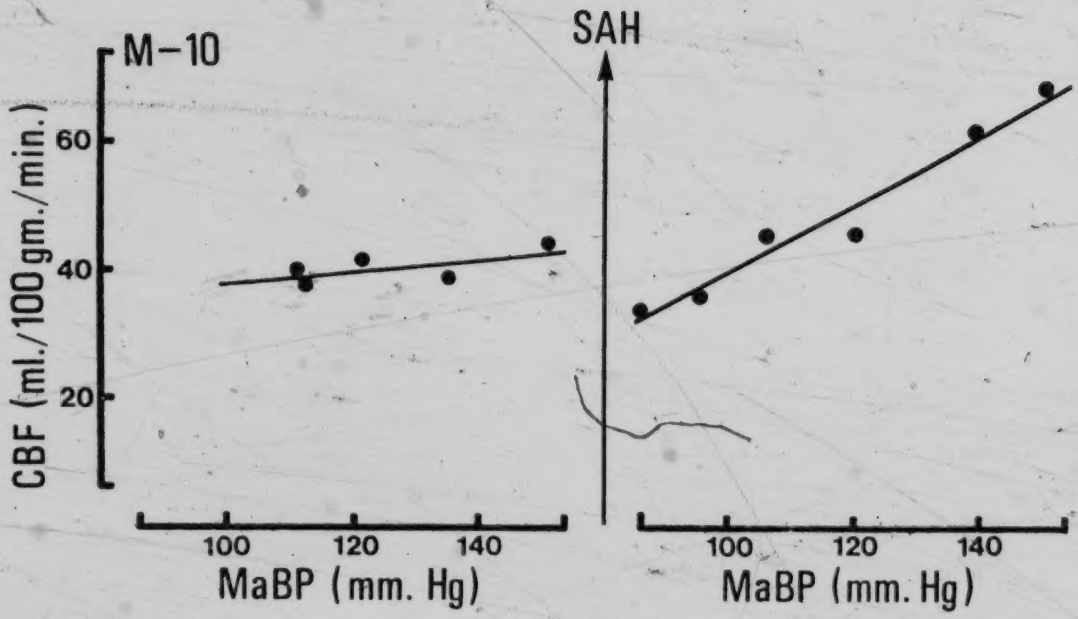
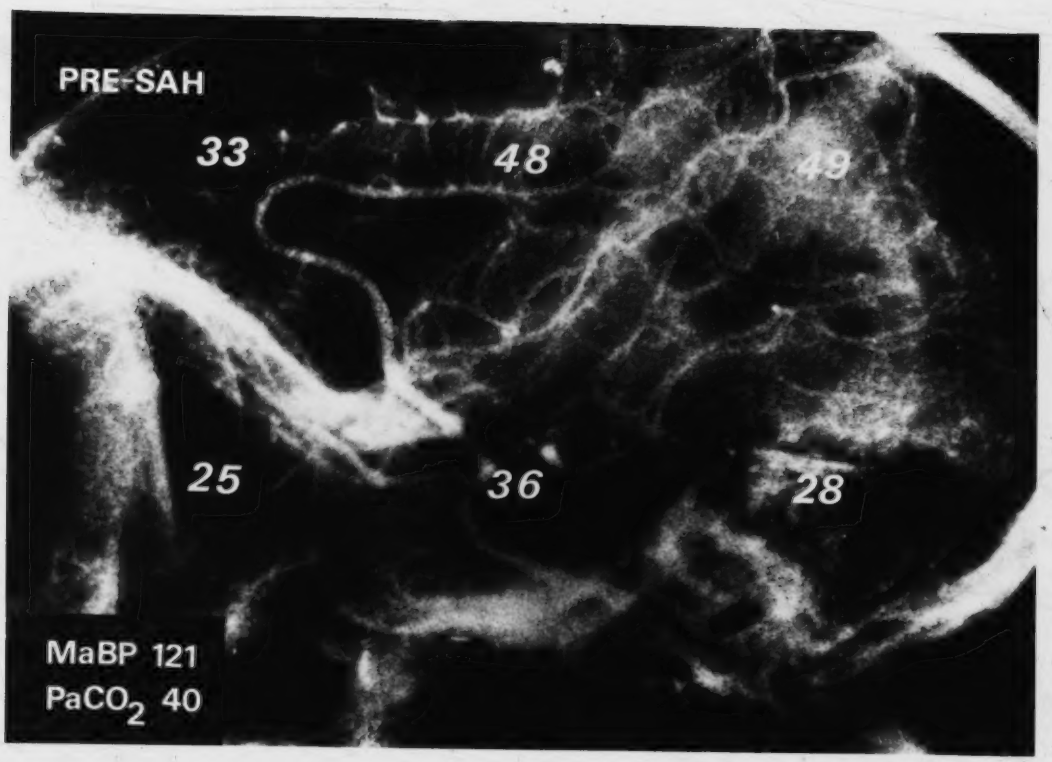
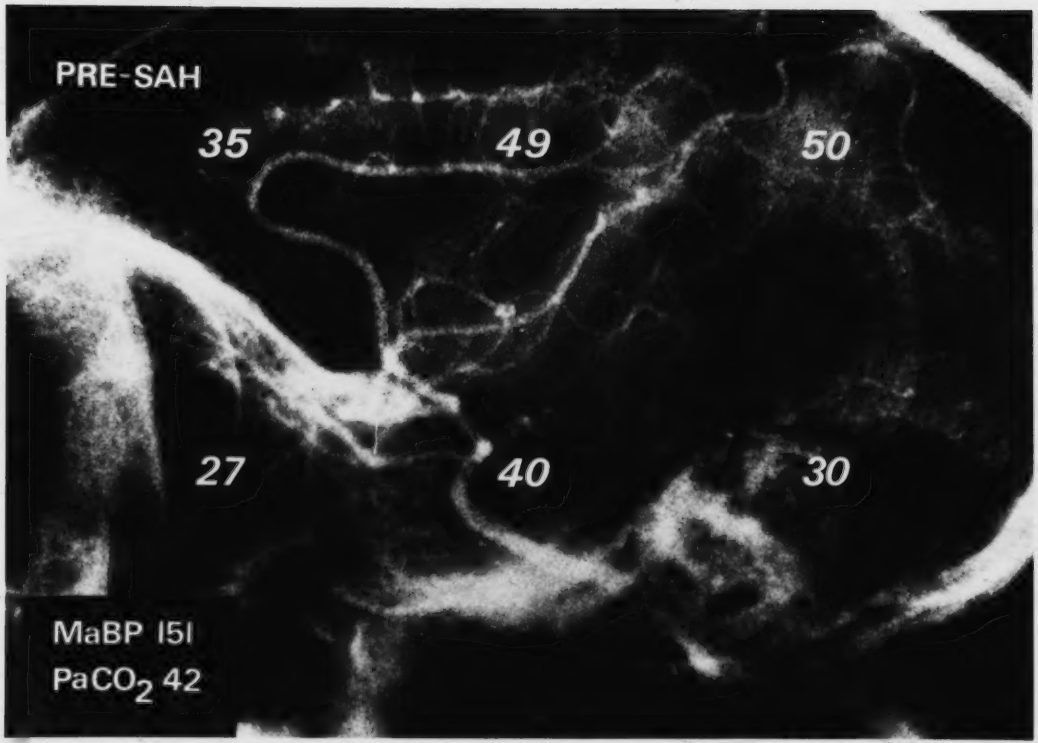


Fig. 8 CBF (mean hemispheric blood flow) responses to increased MaBP. Autoregualtion is impaired following SAH.

Fig. 9 Pre and post-SAH lateral angiograms showing regional CBF values (H/A method) at adifferent blood pressures. A. Pre-SAH during normotension; B. Pre-SAH during hypertension; C. Post-SAH during normotension and D. Post-SAH during hypertension. Note global impairment of CBF autoregulation after SAH.



A



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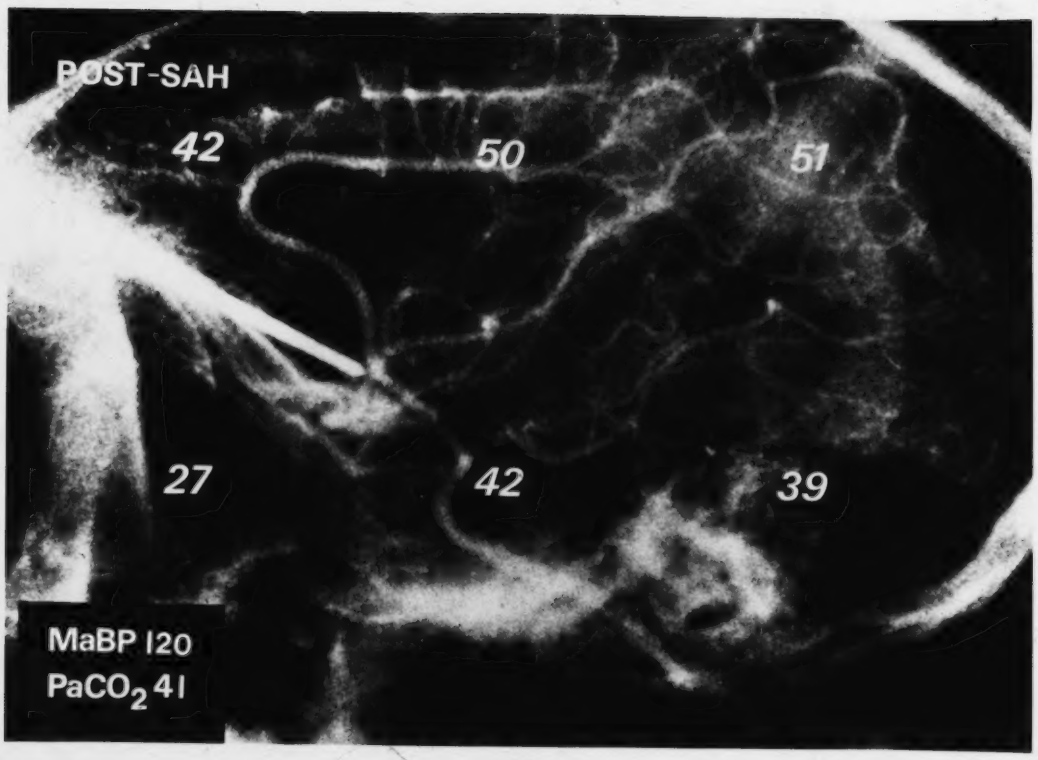

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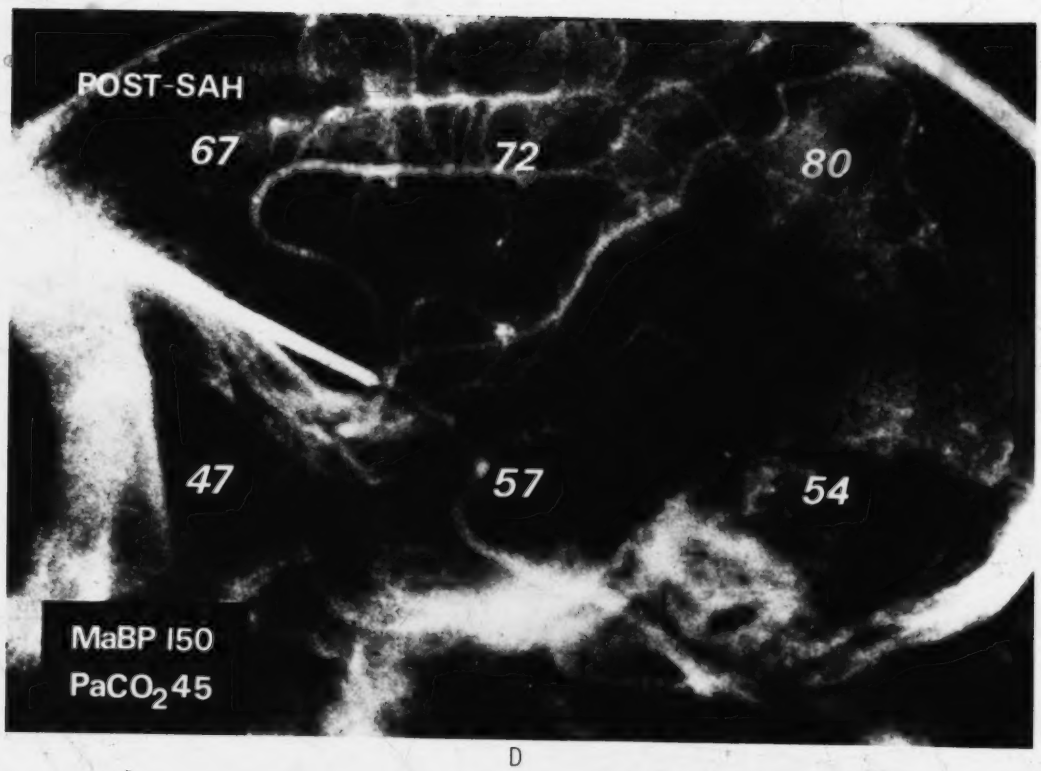


D

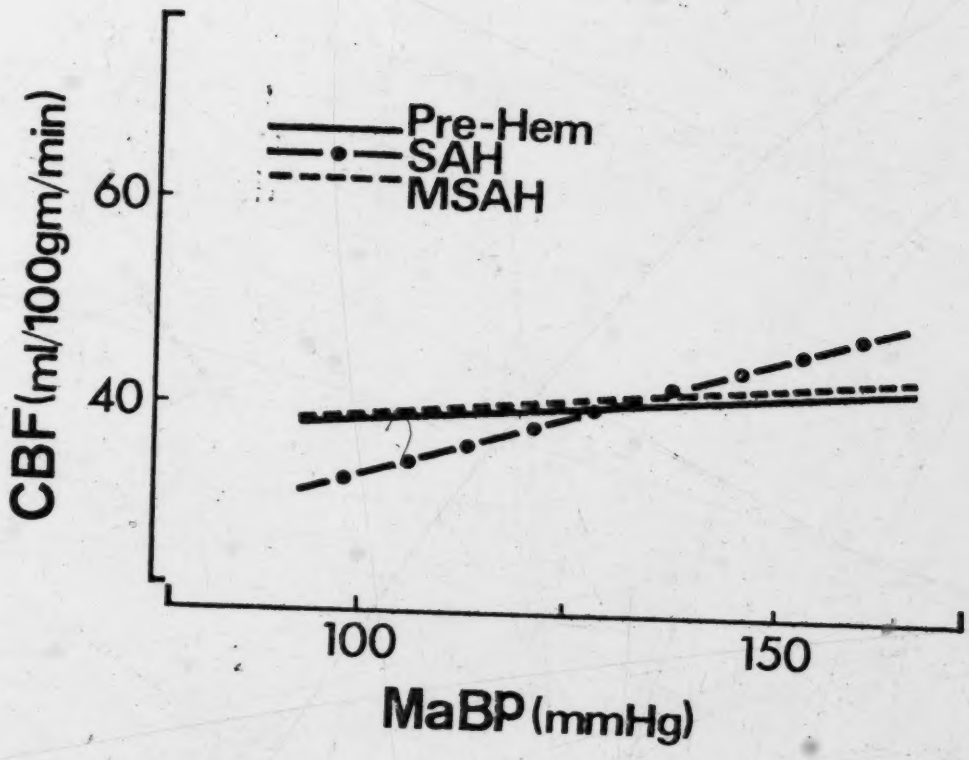


Fig. 10 Linear regression lines showing the relationship between CBF (mean hemispheric blood flow - ISI Method) and mean arterial blood pressure.

Pre-Hem - CBF = 36.8 + 0.05 MaBP

SAH - CBF = 11.1 + 0.23 MaBP

MSAH - CBF = 32.9 + 0.06 MaBP

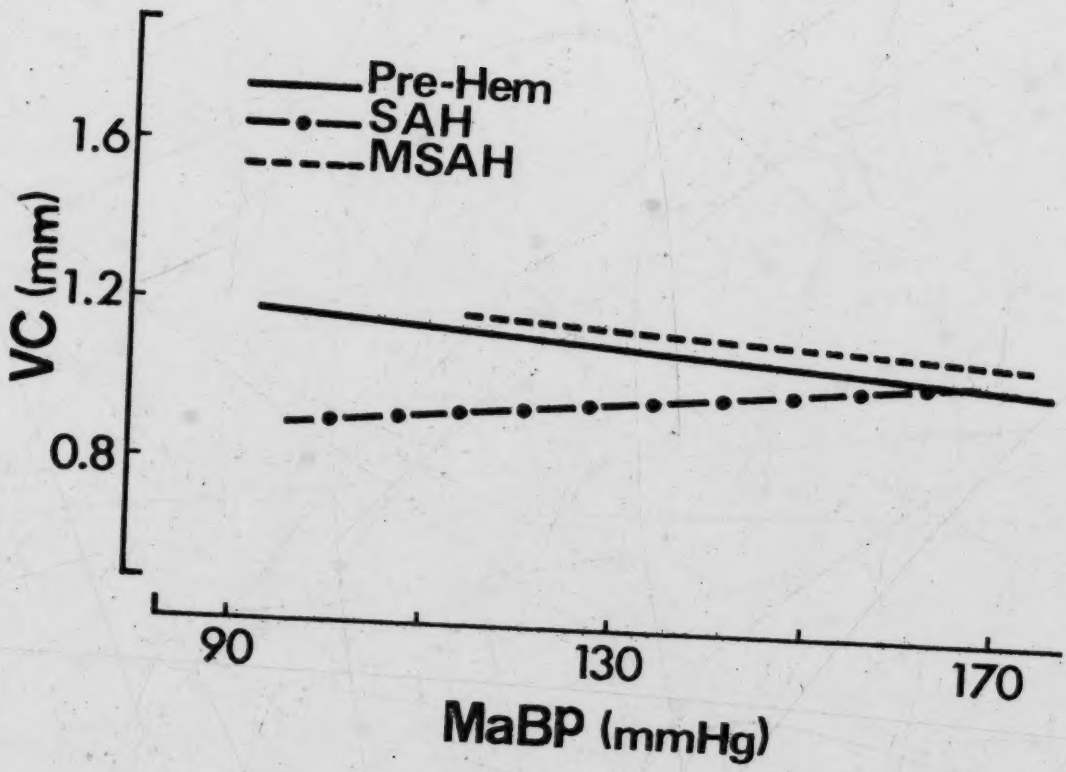


Fig. 11 Linear regression lines showing the relationship between caliber (VC) of the intradural internal carotid artery and mean arterial blood pressure.

Pre-Hem - VC = 1.361 - 0.0018 MaBP

SAH - VC = 0.679 + 0.0013 MaBP

MSAH - VC = 1.381 - 0.0018 MaBP

measurements taken at this site provided the most complete and accurate assessment of cerebral vessel caliber responses to changes in MaBP. Satisfactory angiograms were obtained at different MaBP levels in 13 animals during the pre-hemorrhage period, in 8 animals following SAH, and in 4 animals following MSAH.

During the pre-hemorrhage period the IDICA constricted in response to increases in MaBP; following MSAH the same response was observed. The regression lines for both cases have a negative slope and are not significantly different. Following SAH, IDICA caliber response to an increase in MABP was markedly altered. This regression line showed a positive slope which was significantly different ($p<0.05$) from the slope of the pre-hemorrhage regression line.

(d) Neurological Assessment

Of the 12 animals with SAH, 4 were classed as grade II, 4 grade III, 3 grade IV, and one grade V. There was no clear relationship between neurological grade and degree of cerebral autoregulatory impairment following SAH. Mean post-SAH MaBP/CBF (ISI Method) regression line slope values for grade II, III, and IV animals were 0.33 (±0.08), 0.35 (±0.17), and 0.30 (±0.15) respectively. The animal classed as grade V had a post-SAH regression line slope of 0.13 ml/100g/min/mm Hg.

Two animals with MSAH were classified as grade I, two grade II, and one grade V. There was no apparent cause, found at post-mortem examination, for the poor status of the grade V MSAH animal. In general the animals with MSAH fared better than those with SAH. However, many of the SAH animals were still improving at the time of sacrifice for post-mortem examination of the brain. The 12 SAH animals had essentially 'pure'

subarachnoid hemorrhages as illustrated in Fig. 12. No blood was found in the subarachnoid space of the MSAH animals.

D. Discussion

The presence or absence of arteriographic spasm of the intracranial arteries following rupture of saccular, intracranial aneurysms has long been considered the major factor determining prognosis in these patients. This view has been based on early reports in which a retrospective analysis of patient data was performed. In particular, the reports by Stornelli and French (123) and Allcock and Drake (95) are frequently quoted. In 1973, Robertson (124) stated that "... the leading cause of death and morbidity in the patient with a ruptured intracranial aneurysm who reaches hospital in other than a moribund state is from the effects of cerebral vasospasm."

When considered from a hemodynamic point of view there can be little doubt that cerebral vasospasm, if severe enough, will result in decreased cerebral perfusion, cerebral edema, hypoxia and infarction. However, many clinical studies (125,126,127) have clearly demonstrated the presence of marked vasospasm in the absence of neurological impairment or decreases in cerebral perfusion. In these clinical studies and also in animals studies (128), neurological status has generally correlated best with CBF, whether or not cerebral vasospasm was present. A comprehensive, analytical review of clinical reports dealing with the subject of cerebral vasospasm was recently published by Millikan (97). A total of 46 papers published between 1938 and 1974 were analyzed for evidence that cerebral vasospasm is related to mortality and morbidity in patients with SAH. Finding that no firm evidence of a causal relationship between vasospasm and brain damage was provided by the existing literature, the author performed a study of

Fig. 12 Subarachnoid hemorrhage - basal view

neurological abnormalities in a further series of 198 consecutive patients with acute SAH. The conclusions which were derived from this important study (as presented by the author) were:

(1) There is no clinical picture consistently present coincident with known cerebral vasospasm.

(2) Cerebral vasospasm has no effect on the mortality from SAH due to aneurysm.

(3) There is no relationship between the frequency and severity of the complications from surgical or conservative treatment and the presence or absence of vasospasm.

(4) The prevention of complications must be achieved by increased technical skill with the objective of preventing the cerebral infarction, which is the anatomic substrate for the complication.

Thus, cerebral vasospasm alone is an insufficient condition for development of neurological complications frequently observed in patients with SAH. Other factors, acting alone or perhaps in concert with vasospasm, must therefore be important in this regard.

It has been demonstrated that the efficiency of normal regulatory mechanisms controlling CBF, such as CO_2 reactivity and CBF autoregulation, is impaired in the presence of pathological conditions such as trauma (129), brain tumour (130), and cerebral apoplexy (131), and that this impairment may play an important role in the management of these conditions. Recent studies have provided evidence which suggests that impairment of CBF regulatory mechanisms may similarly occur in patients with SAH.

Kutsuzawa et al (132) tested CBF responses to 5% CO_2 inhalation in 9 patients during the acute period (< 20 days) following SAH and found that the CO_2 vasodilation response was impaired. Subsequent studies in baboons (133) and monkeys (10) have confirmed this observation.

Studies of CBF autoregulation in the presence of SAH have been performed in cats (134), baboons (133) and man (135). Yamaguchi and Waltz (134) performed concurrent measurements of CBF and cortical arterial vessel diameters in cats subjected to SAH induced by middle cerebral artery puncture. CBF was significantly decreased in the ispsilateral hemisphere (same side as punctured artery), and in some cases in the contralateral hemisphere during the measurement period following SAH (1-2 hrs). These decreases in CBF were attributed to spasm of the major vessels (although not visualized)at the base of the brain. Cortical artery measurements in these animals suggested that cerebral autoregulatory responses were impaired by SAH. However, the temporal lobe retraction necessary to perform the arterial puncture may have contributed to the presence of these abnormal vessel responses.

Hashi et al (133) induced SAH in baboons by injection of 10 ml of heparinized blood into the subarachnoid space near the bifurcation of the internal carotid artery. Measurement of cerebral venous outflow were performed during alterations in MaBP induced by inflating and deflating a balloon in the aorta. CBF autoregulation, when tested in this manner at 30-60 minutes following SAH, was noted to be impaired when CPP was lowered but not when it was raised. A state of hyperemia was present when autoregulation was tested at 30-60 minutes following SAH, suggesting that significant cerebral hypoxia occurred during the prolonged elevation in ICP which accompanied the subarachnoid injection of blood (10 minute injection). It is well known that CBF autoregulation to a decrease in CPP is impaired during cerebral hyperemia (85), and that this impairment is probably due to post-hypoxic vasodilation. Twenty-four to 48 hrs after SAH cerebral edema developed and autoregulation was found to be

impaired to both increases and decreases in CPP.

To this author's knowledge, Heilbrun et al (135) have reported the only clinical study of CBF autoregulation following SAH. Global loss of autoregulation was found in 3 of 5 patients tested pre-operatively. All patients had evidence of diffuse ischemia and areas of focal vasoparalysis (impaired autoregulation) as determined by regional CBF measurements with ^{133}Xe. In 8 post-operative patients focal vasoparalysis was present in 6, 2 of these with global loss of autoregulation. Interpretation of these results is made difficult by the fact that hydrocephalus (angiographic evidence) was frequently present in these patients, suggesting the possibility of decreased CPP below the autoregulatory range. Autoregulation was tested by lowering the MaBP in the pre-operative period. In the post-operative period autoregulation was tested either by lowering or raising the blood pressure. However, no information is provided regarding which method was used in individual patients.

On the basis of the above studies it can be concluded that no clear evidence of impaired autoregulation following SAH has been presented. The present study was carried out in an attempt to resolve this important question. Rhesus monkeys were utilized because of the similarity between the cerebral circulation in these animals and humans. The hypertensive agent used (Aramine) to test autoregulation has previously been shown to have no effect on normal cerebral autoregulatory responses (136).

The animals used in the present study demonstrated efficient pre-hemorrhage autoregulatory responses which maintained cerebral perfusion relatively constant in the presence of moderate increases in blood pressure. Furthermore, these increases in MaBP produced vasoconstriction of the large cerebral arteries and were without effect on CSF pressure.

Therefore, the cerebral arterial responses obtained prior to subarachnoid injection of blood or artificial CSF were unimpaired by the experimental preparation.

Subarachnoid hemorrhage was induced over a short period of time which resulted in CSF pressure increases similar to those reported in patients experiencing a SAH (93). Following subarachnoid injection of blood, CSF pressure remained elevated at normotension and when MaBP was subsequently increased to test CBF autoregulation, concurrent further increases in CSF pressure occurred. There is experimental evidence which indicates that, when total "vasomotor paralysis" has developed,a completely passive relationship exists between ICP and MaBP and large increases in ICP result from moderate increases in MaBP (137). The factors responsible for the increase in ICP appear to be acute cerebral edema and passive dilatation of cerebral vessels (138). In the present study, cerebral arterial caliber measurements demonstrated that arterial dilatation occurred in response to increases in MaBP after SAH, indicating that increased vascular volume was at least partly responsible for the elevation in ICP.

Increases in CSF pressure with elevation of MaBP will tend to prevent an increase in CBF and may lead to an under estimation of the degree of autoregulatory impairment when MaBP is used as an index of cerebral perfusion pressure. It is evident that this occurred to some degree in the present study. However, analysis of the post-SAH relationship between CBF and MaBP still revealed significant impairment of CBF autoregulation.

It was considered possible that a subarachnoid injection causing a large transient increase in CSF pressure could of itself cause impaired cerebral autoregulatory responses. Therefore, a control group of animals was tested in which artificial CSF was injected into the subarachnoid

space. Although these animals experienced CSF pressure increases similar to those measured for the SAH group, the CBF, arterial caliber and CSF pressure responses to increased MaBP all remained normal. Thus, the changes in cerebral arterial reactivity observed with SAH were not related to an increase in CSF pressure but rather to the presence of blood in the subarachnoid space. Shannon et al (139) and Sugi et al (140) have reported increases in CSF lactate and pyruvate and decreases in CSF bicarbonate and pH within a few hours following injection of blood into the subarachnoid space in animals. Similar CSF acid-base and metabolic changes have been described in patients with SAH (141,142). Such increases in CSF acid metabolites occur following ischemia or hypoxia and are considered to be responsible for impairment of CBF autoregulation (143,79). However, there is experimental evidence which indicates that similar increases in CSF acidity can originate from glucose metabolism of blood cells in CSF and presumably also lead to impaired autoregulation (144, 139).

The abnormal cerebrovascular responses observed following SAH in the present study are in agreement with recent clinical observations in patients with SAH. Hayashi et al (145) observed intracranial "pressure waves" which were related to periodic breathing of the Cheyne-Stoke type and variations in arterial pressure. The results of other investigations indicate that such repeated elevations in intracranial pressure may eventually lead to complete "vasomotor paralysis" and severe sustained intracranial hypertension. Indeed, the authors found that the more seriously ill patients demonstrated ICP levels in excess of 1000 mm H_2O and total loss of normal cerebrovascular reactivity. Similarly, Sakurai et al (146), in testing CBF autoregulation and response to hypercapnia in patients with SAH, found a close relationship between degree of

impaired reactivity and neurological condition.

The precise conditions under which cerebral vasospasm contributes to the development of cerebral ischemia have not been elucidated. Martins et al (147) have suggested that the interplay of many factors, including vasospasm, brain edema and impaired autoregulation determines cerebral perfusion after SAH. Similarly, our results support the concept that the effect of vasospasm on CBF will depend in large measure on the status of autoregulation, intracranial pressure and systemic blood pressure. However, the manner in which vasospasm and these factors are related in determining cerebral perfusion can only be postulated.

With normal reactivity and perfusion pressure, distal compensatory vasodilation can maintain normal CBF even in the presence of severe arterial constriction. Further dilatation to compensate for a fall in perfusion pressure however, is impaired (148). Thus, a precarious state of "compensated vasospasm" may develop in SAH patients and ischemia may be precipitated by systemic hypotension or increased ICP. On the other hand, the occurrence of severe vasospasm in a non-regulating vascular bed may produce ischemia directly due to lack of distal compensatory dilatation. Again, the ischemia potential of the vasospasm under these conditions is enhanced when associated with increased ICP or decreased MaBP. It is evidence that, even without vasospasm, the combination of severely impaired cerebrovascular reactivity and a fall in perfusion pressure can produce critically low levels of CBF.

These relationships indicate the importance of MaBP and ICP in determining cerebral perfusion in patients with SAH and impaired autoregulation, whether or not significant vasospasm has developed. Moreover, they suggest that increases in blood pressure may improve cerebral perfusion, particularly in patients who develop hypotension and/or vaso-

spasm. It must be emphasized, however, that there is a potential danger to the brain from repeated or sustained increases in blood pressure if this results in severe concurrent increases in ICP.

II. STUDY B. THE EFFECT OF SUBARACHNOID BLOOD AND SEROTONIN ON rCBF, CEREBRAL VESSEL CALIBER, NEUROLOGICAL STATUS AND CEREBRAL ARTERY ULTRASTRUCTURE.

A. Objectives

This study comprises three-parts: In-vitro pharmacological experiments; in-vivo experiments; and electron microscopy examination of cerebral vessels. The objectives of each portion of the study are listed below.

(a) In vitro experiments

(i) To determine the physiological concentration of serotonin in monkey serum.

(ii) To assess the ability of cyproheptadine to block serotonin and serum induced contractions in monkey cerebral vessels.

(b) In vivo experiments

(i) To quantitatively determine the effect of a subarachnoid injection of artificial cerebrospinal fluid on rCBF, cerebral vessel caliber and other physiological parameters in a control group of cynmolgous monkeys.

(ii) To quantitatively determine the effect of a subarachnoid injection of blood and physiological concentrations of serotonin on rCBF, cerebral vessel caliber and other physiological parameters in the cynmolgous monkey.

(iii) To determine and compare the effect of the specific serotonin antagonist, cyproheptadine, on subarachnoid blood and serotonin induced changes in rCBF and cerebral vessel caliber.

(iv) To assess the relationship between changes in cerebral vessel caliber and rCBF.

(v) To assess the duration of changes in rCBF and cerebral vessel caliber following subarachnoid injection of blood and serotonin.

(vi) To determine the effect of cerebral circulatory changes induced by subarachnoid blood and serotonin on neurological status.

(c) Electron microscopy

(i) To determine the effect of cerebral circulatory changes induced by subarachnoid blood and serotonin on the ultrastructure of cerebral vessels at the arterial, arteriolar and capillary levels.

B. Experimental Design

In-vitro experiments were carried out to obtain an estimate of the normal concentration of serum serotonin in monkey serum. The results of these experiments determined the concentration of the serotonin solution to be injected into the subarachnoid space in the in-vivo experiments. A second set of in-vitro experiments was conducted to verify the ability of cyproheptadine to block serotonin and serum induced contractions in monkey cerebral arteries.

The experimental design for the in-vivo experiments consisted of 3 major experimental categories which were established on the basis of the type of subarachnoid injection to be performed; blood, serotonin and artificial CSF. Each major category was then subdivided into one group which would receive cyproheptadine and one group which would not receive

cyproheptadine. The 6 treatment categories which were thus established are listed below.

1. Subarachnoid Hemorrhage (SAH)
2. SAH followed by serotonin blocking agent* (SAH-CH)
3. Subarachnoid serotonin injection (SSI)
4. SSI followed by serotonin blocking agent (SSI-CH)
5. Subarachnoid artificial CSF injection (MSAH)
6. MSAH followed by serotonin blocking agent (MSAH-CH)

* I.V. Cyproheptadine 1.0 mg/kg.

These treatment categories were assigned to randomized blocks, each randomized block containing the 6 treatment categories in random order. Experiments were then performed according to this design. A total of 30 monkeys were utilized in this series of experiments, each treatment group containing five animals.

Experimental protocols utilized are summarized below.

Treatment categories 1, 3 and 6:

Surgery → Cerebral angiography → 2-3 rCBF measurements → Cerebral Angiography → Subarachnoid injection of blood, serotonin solution, or artificial CSF → 2-3 rCBF measurements → Cerebral angiography → 4-6 rCBF measurements → Cerebral angiography → Reverse anesthetic → Neurological assessment → Post-mortem examination

Treatment Categories 2, 4 and 5:

Surgery → Cerebral angiography → 2-3 rCBF measurements → Cerebral angiography → Subarachnoid injection of blood, serotonin solution or artificial CSF → 2-3 rCBF measurements → Cerebral angiography → Serotonin blocking agent → 4-6 rCBF measurements → Angiography → Reverse anesthetic → Neurological assessment → Post-mortem examination

The major variables measured were rCBF, cerebral vessel caliber, cerebral perfusion pressure (MaBP-CSFP) and neurological status (2 hrs and 16-18 hrs post-anaesthesia). Secondary variables measured included $PaCO_2$, PaO_2, pH, heart rate (HR) and hematocrit (Hct).

Following completion of the above experiments, an additional 4 monkeys were utilized in which the concentration of the serotonin solution injected into the subarachnoid space was increased ten-fold. The experimental protocol utilized was slightly different for these animals in that cerebral angiography was also performed immediately following subarachnoid injection and only 2-3 rCBF measurements were done prior to taking the final angiograms. These animals did not receive cyproheptadine.

After the final neurological assessment 18 of the initial 30 monkeys were re-anaesthetized by intravenous injection of sodium pentothal and perfused with glutaraldehyde. This group comprised 6 animals with SAH, 7 with SSI and 5 with MSAH. Cerebral vessels obtained from these animals were examined by electron microscopy for evidence of ultrastructural changes.

C. Results

(a) In-vitro experiments

A bioassay determination of the serotonin concentration in monkey serum is shown in Fig. 13. The dose-response lines for serum and serotonin are parallel over the concentration values tested. The equivalent serotonin concentration for 9% serum is 5.6×10^{-7} M and for 100% serum is 6.2×10^{-6} M. Values for all such serotonin bioassay determinations are given in Table 2. The mean serotonin concentration values (1.6×10^{-5} M) for cynmolgous monkey serum was higher than the mean value for rhesus monkey serum. This discrepancy, however, was due to a very high value obtained from one cynmolgous monkey (C-1). The mean concentration value obtained for cynmolgous monkey serum is 7.4×10^{-6} M when C-1 is excluded. The median value for the cynmolgous group is 6.3×10^{-6} M. On the basis of these results a concentration of 5×10^{-6} M for serotonin was defined as a physiological concentration. Thus, in subsequent in-vivo experiments, a solution (artificial CSF) containing 5×10^{-6} M serotonin was used for subarachnoid injections (with the exception of a separate group of 4 animals in which 5×10^{-5} M serotonin was used.)

A second series of in-vitro experiments were conducted in which cyproheptadine was used to block serotonin and serum-induced contractions in monkey (cynmolgous) internal carotid (ICA) and basilar arteries (BA). Serum and serotonin (5×10^{-6} M) induced contractions were completely blocked by 5×10^{-6} M cyproheptadine (Fig. 14). Subsequent injection of the prostaglandin $F_{2\alpha}$ into the organ bath demonstrated that the arterial preparations were still capable of vigorous contraction. Serotonin dose-response curves were determined with and without cyproheptadine in 2

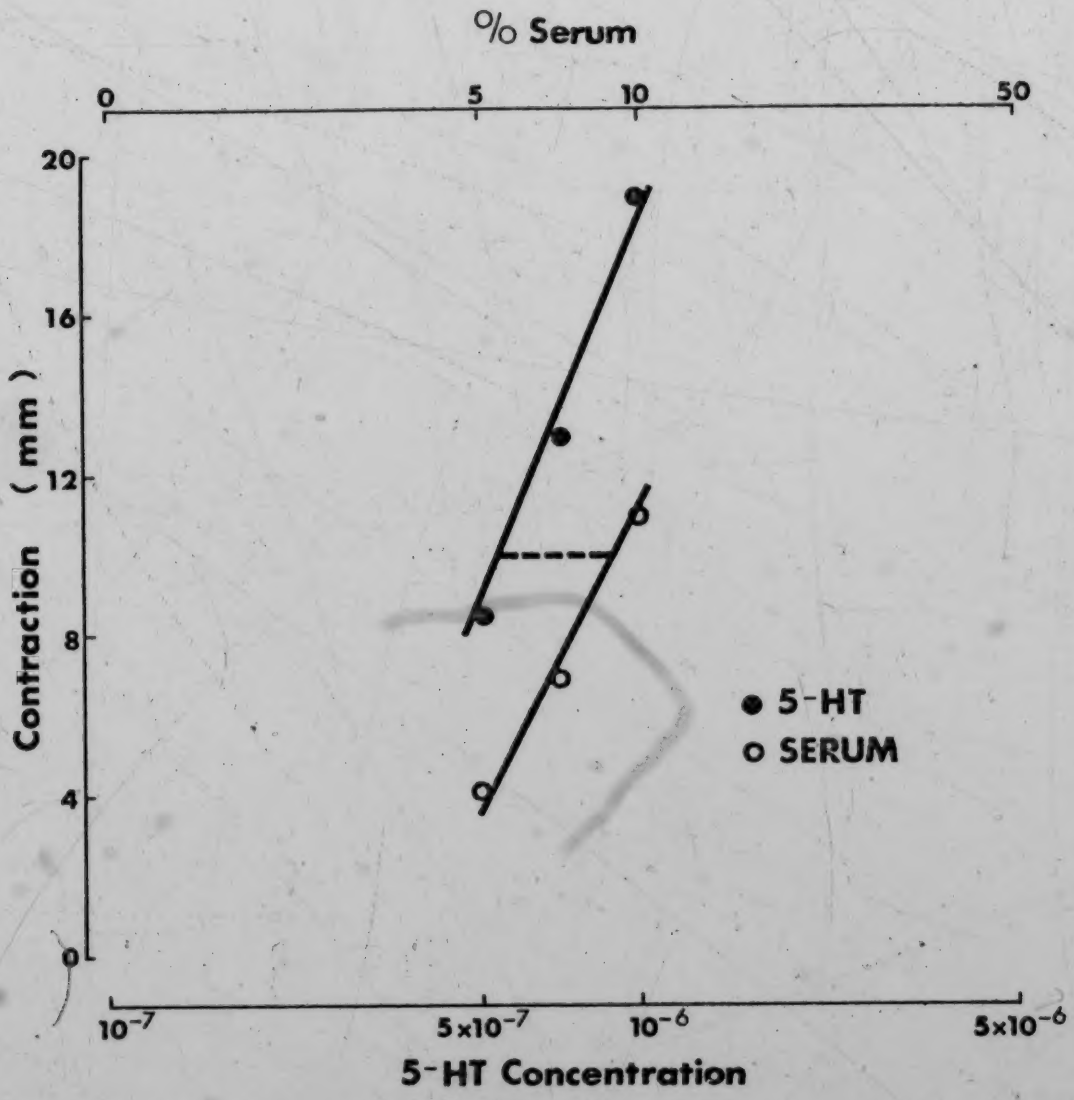


Fig. 13 Example of a bioassay determination of monkey serum serotonin concentration. Dashed line joins serum concentration equivalent to known serotonin (5-HT) concentration.

TABLE 2

RESULTS OF BIOASSAY OF MONKEY SERUM SEROTONIN CONCENTRATIONS

Serum	Tissue	Molar Concentration of Serum 5-HT	
R-1	Umbilical Artery		1.4×10^{-5}
R-2	Basilar Artery	2.5×10^{-6}	2.2×10^{-6}
R-2	Middle Cerebral Artery	2.0×10^{-6}	
R-3	Umbilical Artery	3.5×10^{-6}	5.0×10^{-6}
R-3	Umbilical Artery	6.2×10^{-6}	
R-3	Umbilical Artery	5.3×10^{-6}	
R-4	Basilar Artery	1.2×10^{-5}	7.6×10^{-6}
R-4	Umbilical Artery	2.3×10^{-6}	
R-4	Umbilical Artery	8.4×10^{-6}	
R-5	Umbilical Artery		1.1×10^{-5}
		mean =	8.0×10^{-6}
C-1	Umbilical Artery		6.9×10^{-5}
C-2	Umbilical Artery		6.3×10^{-6}
C-3	Umbilical Artery		1.1×10^{-5}
C-4	Umbilical Artery		3.4×10^{-6}
C-5	Umbilical Artery		1.7×10^{-5}
C-6	Stomach Fundus	2.8×10^{-6}	3.2×10^{-6}
C-6	Stomach Fundus	3.6×10^{-6}	
C-7	Stomach Fundus	3.8×10^{-6}	3.4×10^{-6}
C-7	Stomach Fundus	3.0×10^{-6}	
		mean =	1.6×10^{-5}
		overall mean =	1.3×10^{-5}

R - Rhesus

C - Cynmolgous

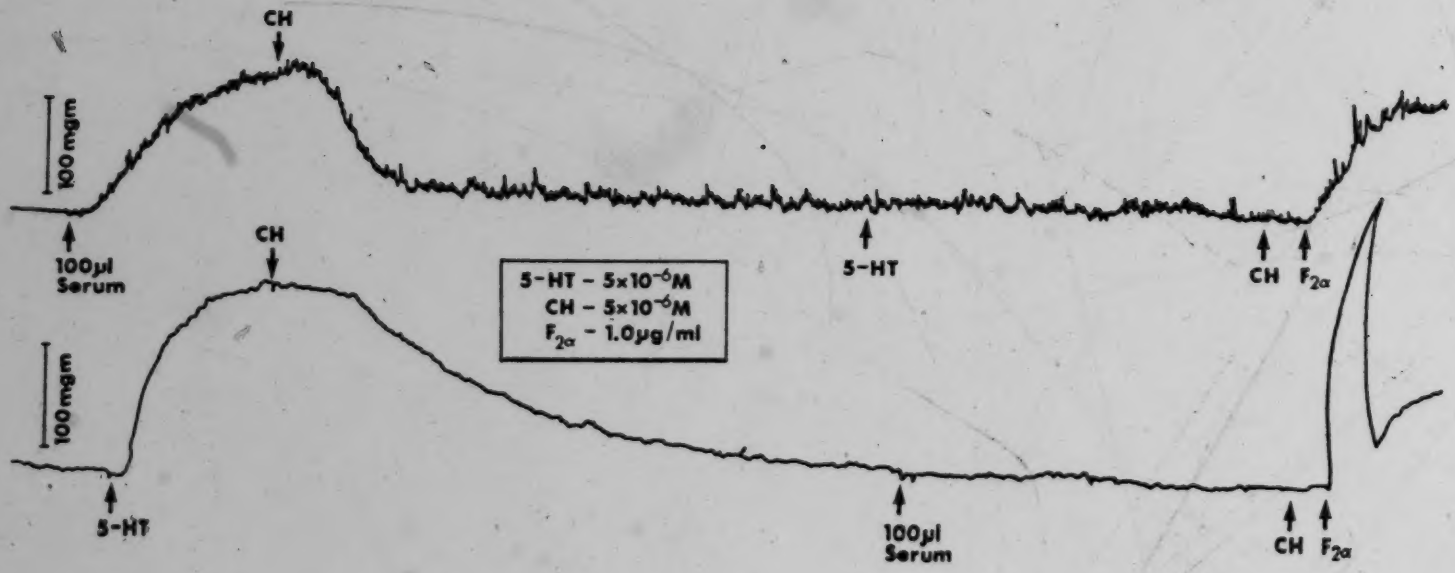


Fig. 14 Effect of cyproheptadine (CH) on the contraction of a monkey isolated internal carotid (ICA) and basilar artery (BA) induced by serotonin (5-HT) or monkey serum.

vessels (Fig. 15). Cyproheptadine (5×10^{-6} M) completely blocked the response to serotonin in both the ICA and BA. In 5 ICA and 4 BA preparations, the percentage maximal contraction with 5×10^{-6} M serotonin was 84.3 (±16.2) and 93.5 (±6.2) respectively. In the presence of 5×10^{-6} M cyproheptadine the corresponding values were 2.3 (±3.6) and 0.2 (±0.5).

(b) In-vivo experiments

Physiological data values from the 30 experiments done according to a randomized block design are presented according to treatment in Table 3 and Table 4. This data is further subdivided into three groupings:

Group I - Pre-subarachnoid injection data

Group II - Post-subarachnoid injection data obtained prior to the first post-injection angiograms.

Group III - Post-subarachnoid injection data obtained following the first post-injection angiograms or I.V. administration of cyproheptadine.

These results demonstrate that physiological stability was maintained in the course of these experiments.

Peak CSF pressures (mm Hg) observed during subarachnoid injection of 3 cc of blood, serotonin solution, or artificial CSF were 126±36 (n=7), 121 ± 33 (n=10), and 129 ± 52 (n=7) respectively. These increases in CSFP were often accompanied by increases in MaBP, bradycardia, and EKG changes. Premature ventricular contractions, S-T elevations, T-wave inversions, and arrythmias were frequently noted. EKG pattern and MaBP usually returned to normal in 3-5 minutes. CSFP remained elevated following SAH but returned to normal levels following SSI and MSAH.

(i) MSAH - Mock Subarachnoid Hemorrhage

The effects of MSAH on CBF (mean hemispheric blood flow), cerebral

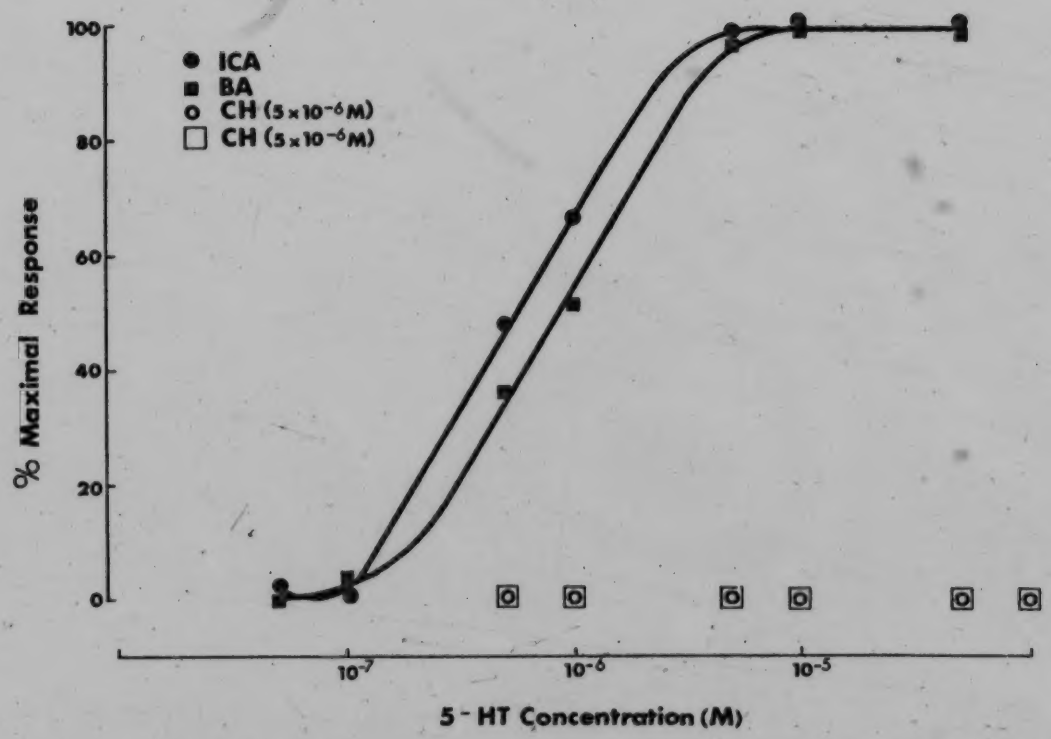


Fig. 15 Serotonin (5-HT)-induced contraction of a monkey isolated internal carotid (ICA) and basilar artery (BA) in the presence and absence of cyproheptadine (CH).

TABLE 3

EXP.	A	B	C	D	E	F
$PaCO_2$						
Group 1	41 ± 1	41 ± 1	41 ± 2	42 ± 2	41 ± 1	40 ± 2
Group 2	38 ± 1	40 ± 1	40 ± 0	39 ± 1	39 ± 1	38 ± 1
Group 3	39 ± 1	39 ± 0	40 ± 1	40 ± 2	39 ± 1	38 ± 1
PaO_2						
Group 1	135 ± 2	129 ± 2	128 ± 3	125 ± 2	130 ± 4	134 ± 3
Group 2	134 ± 1	135 ± 6	130 ± 0	131 ± 5	132 ± 3	134 ± 2
Group 3	135 ± 1	141 ± 14	127 ± 1	128 ± 3	132 ± 7	132 ± 2
PH						
Group 1	7.42 ± 0.01	7.44 ± 0.01	7.38 ± 0.01	7.40 ± 0.03	7.42 ± 0.02	7.44 ± 0.02
Group 2	7.42 ± 0.01	7.42 ± 0.01	7.39 ± 0.01	7.43 ± 0.02	7.42 ± 0.01	7.44 ± 0.02
Group 3	7.44 ± 0.01	7.43 ± 0.01	7.38 ± 0.01	7.41 ± 0.02	7.42 ± 0.02	7.42 ± 0.01

Arterial blood gas and pH values during CBF Measurements (mean ± S.D.)

TABLE 4

EXP.	A	B	C	D	E	F
MBP						
Group 1	110 ± 4	108 ± 2	112 ± 6	106 ± 1	110 ± 5	110 ± 1
Group 2	108 ± 4	112 ± 4	110 ± 1	102 ± 3	109 ± 5	107 ± 3
Group 3	106 ± 3	119 ± 4	107 ± 4	107 ± 3	109 ± 6	102 ± 2
ICP						
Group 1	10 ± 0	8 ± 0	9 ± 1	11 ± 0	10 ± 1	9 ± 1
Group 2	18 ± 2	14 ± 1	11 ± 1	10 ± 0	12 ± 3	11 ± 1
Group 3	19 ± 1	19 ± 1	10 ± 1	12 ± 2	13 ± 4	10 ± 1
HCT						
Group 1	40 ± 1	38 ± 1	38 ± 1	41 ± 1	40 ± 1	39 ± 1
Group 2	39 ± 0	39 ± 1	39 ± 0	41 ± 1	40 ± 1	39 ± 1
Group 3	37 ± 0	39 ± 0	40 ± 0	41 ± 1	40 ± 1	40 ± 1
HR						
Group 1	213 ± 5	217 ± 4	229 ± 4	216 ± 5	221 ± 6	221 ± 3
Group 2	216 ± 5	224 ± 6	232 ± 10	209 ± 3	221 ± 8	220 ± 4
Group 3	218 ± 2	223 ± 7	231 ± 2	216 ± 8	223 ± 7	223 ± 3

Arterial blood pressure (MaBP), cerebrospinal fluid pressure (CSFP), hematocrit (Hct) and heart rate (HR) values during CBF measurements (mean ± S.D.)

vessel caliber (IDICA) and CPP are shown in Fig. 16. Cerebral perfusion remained at or near pre-MSAH levels during the 2-2½ hr period following MSAH. Cyproheptadine administration in 5 of the 10 animals at approximately 1 hr after MSAH had no effect on CBF. Similarly, cerebral vessel caliber (IDICA) was unchanged following MSAH in 10 animals or following subsequent cyproheptadine administration in 5 of these animals. CPP remained constant except for a 10-15% decrease during the latter part of the experiments in the group of 5 animals without cyproheptadine.

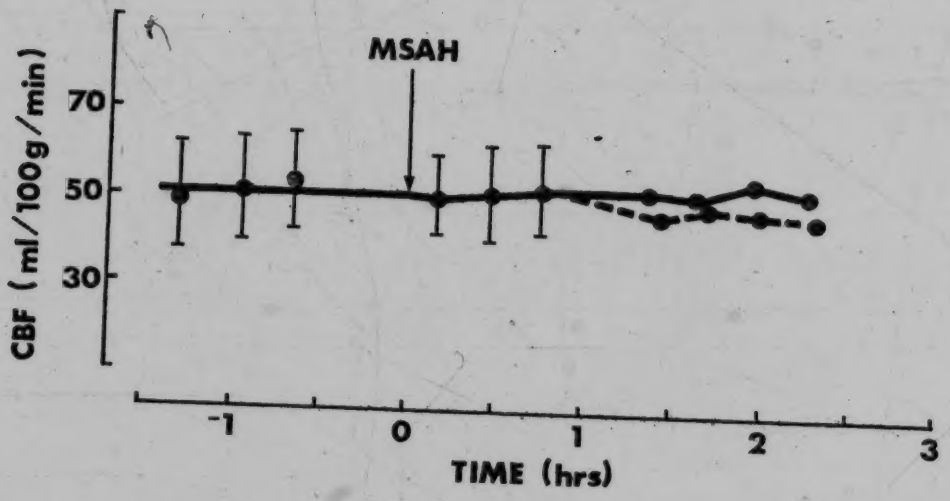
(ii) SAH-Subarachnoid Hemorrhage

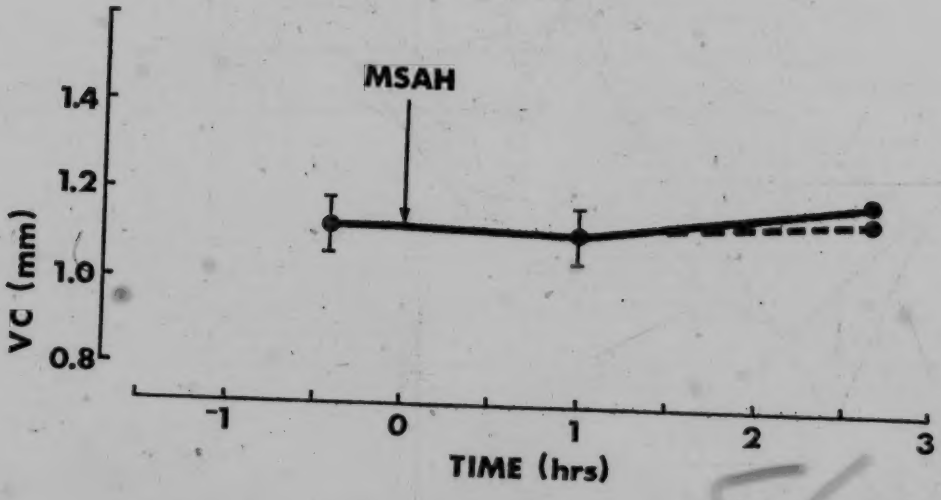
The effects of SAH on CBF (mean hemispheric blood flow), cerebral vessel caliber (IDICA) and CPP are shown in Fig. 17. Following SAH a significant but transient decrease in CBF occurred within 1 hr cerebral perfusion had returned to pre-hemorrhage levels and subsequently remained essentially unchanged whether or not cyproheptadine was administered.

CBF changes, whether spontaneous or induced by SAH, invariably occurred simultaneously and almost equally in all 4 cerebral regions monitored. This finding is shown for one animals in which a SAH was performed (Fig. 18).

IDICA caliber was significantly decreased at 1 hr following SAH and at 2½-3 hrs in the animals not receiving cyproheptadine. IDICA caliber in animals receiving cyproheptadine was not significantly different from the pre-SAH value; however, the recovery in IDICA caliber observed following cyproheptadine was minimal. Although all animals demonstrated some post-SAH decrease in IDICA caliber, only 3 had decreases in excess of 25%. The cerebral vasospasm observed in one of these animals is shown in Fig. 19. In this animal, the percent decrease in IDICA, MCA, PPA and DPA caliber at 1 hour post-SAH was 27, 42, 34 and 23 respectively; at 2½ hrs the corresponding values were 30, 23, 29 and 20. Thus, only the MCA showed

Fig. 16 Mean and standard deviation of CBF (mean hemispheric blood flow), intradural internal carotid artery (IDICA) caliber and cerebral perfusion pressure (CPP) for 10 animals subjected to subarachnoid injection of artificial cerebrospinal fluid (3 ml.). Dashed lines connect values for 5 of the 10 animals which received intravenous cyproheptadine (1 mg/kg).


MSAH
CBF (ml/100g/min)
70
50
30
-1
0
1
2
3
TIME (hrs)


MSAH
VC (mm)
1.4
1.2
1.0
0.8
-1
0
1
2
3
TIME (hrs)

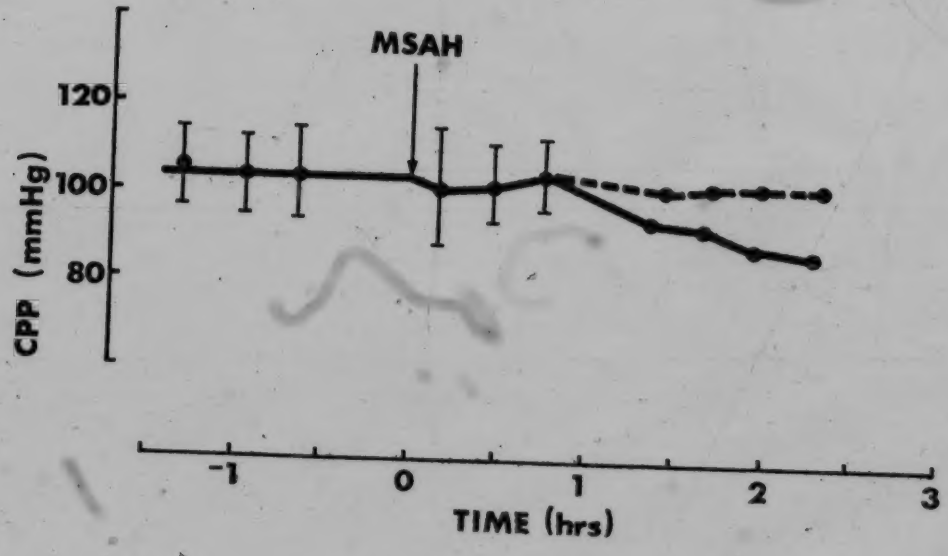
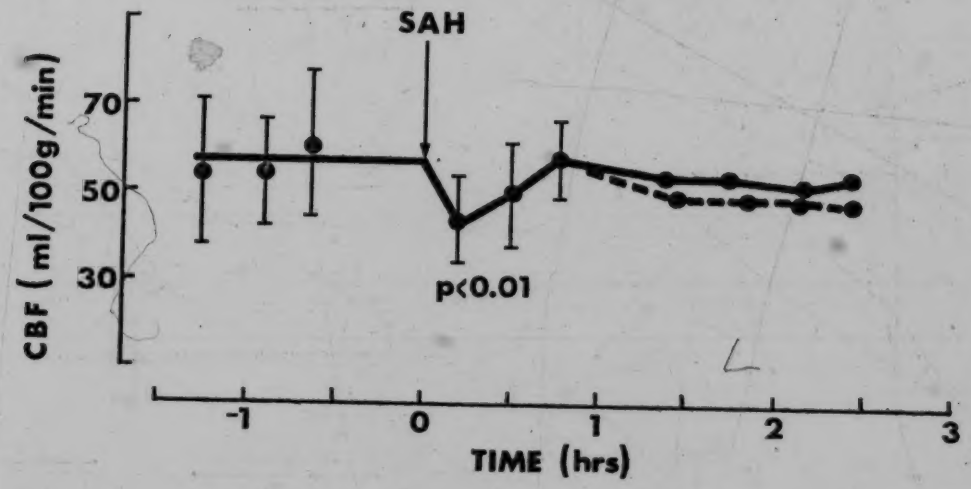
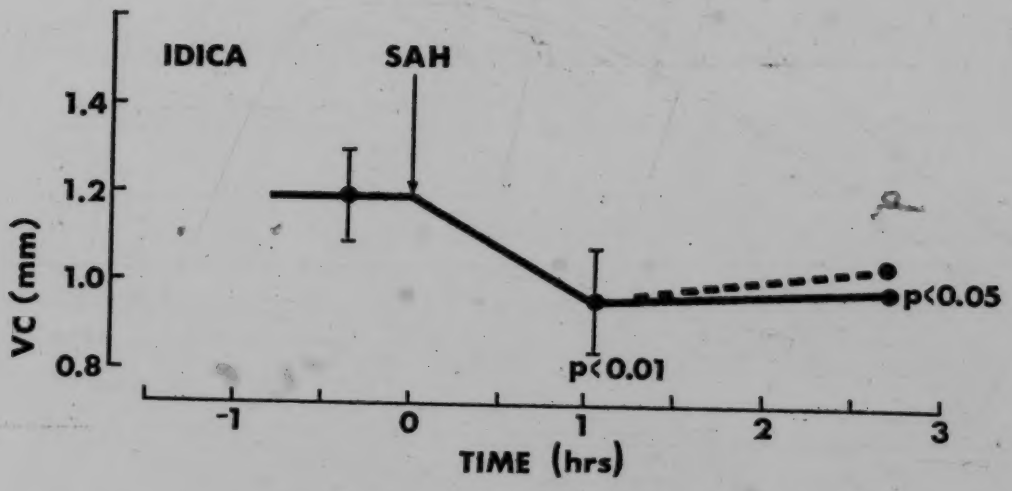
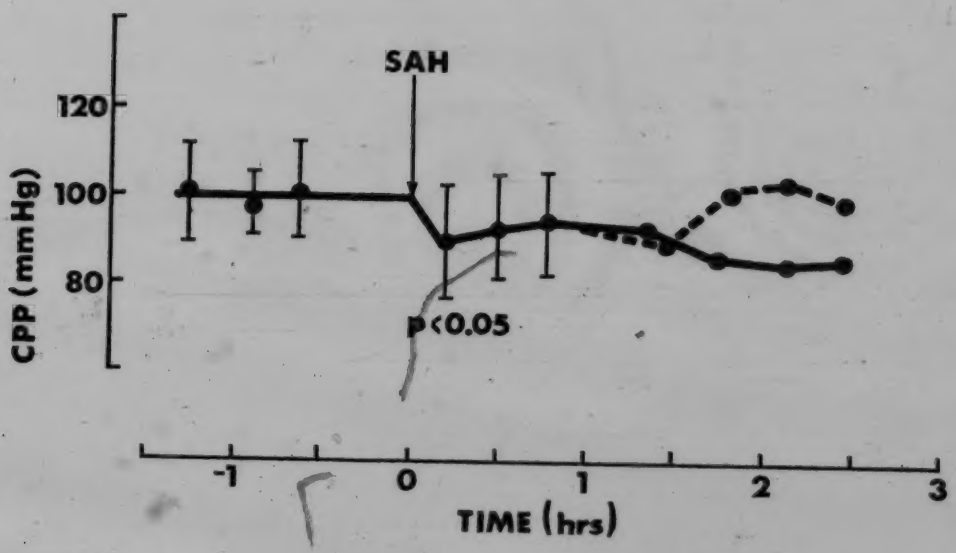

MSAH
CPP (mmHg)
120
100
80
-1
0
1
2
3
TIME (hrs)

Fig. 17 Mean and standard deviation of CBF (mean hemispheric blood flow), intradural internal carotid artery (IDICA) caliber and cerebral perfusion pressure (CPP) for 10 animals subjected to subarachnoid injection of blood (3 ml.). Dashed lines connect values for 5 of the 10 animals which received intravenous cyproheptadine (1 mg/kg).


SAH
CBF (ml/100g/min)
70
50
30
p<0.01
-1
0
1
2
3
TIME (hrs)


IDICA
SAH
VC (mm)
1.4
1.2
1.0
0.8
p<0.05
p<0.01
-1
0
1
2
3
TIME (hrs)


SAH
CPP (mmHg)
120
100
80
p<0.05
-1
0
1
2
3
TIME (hrs)

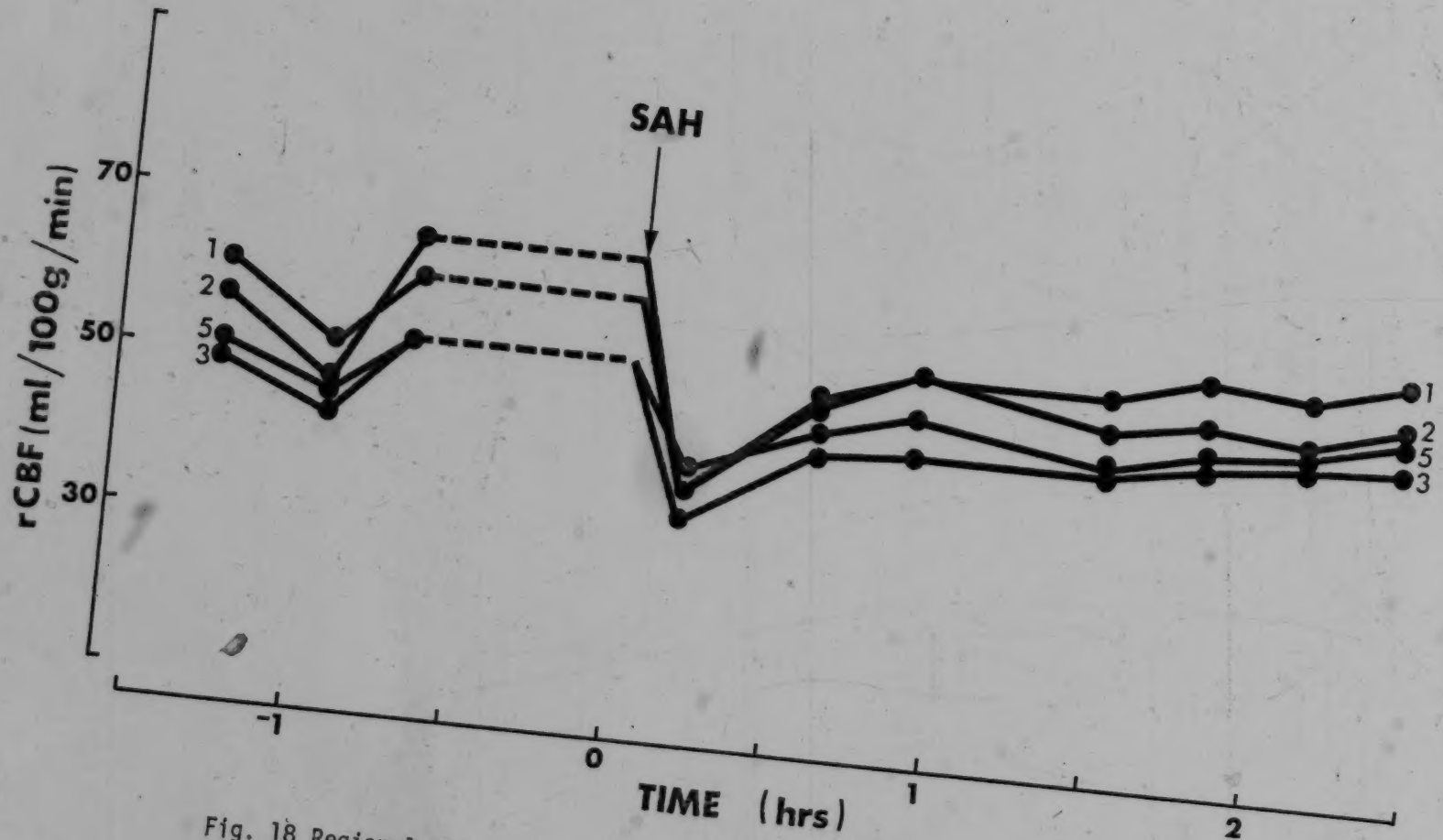


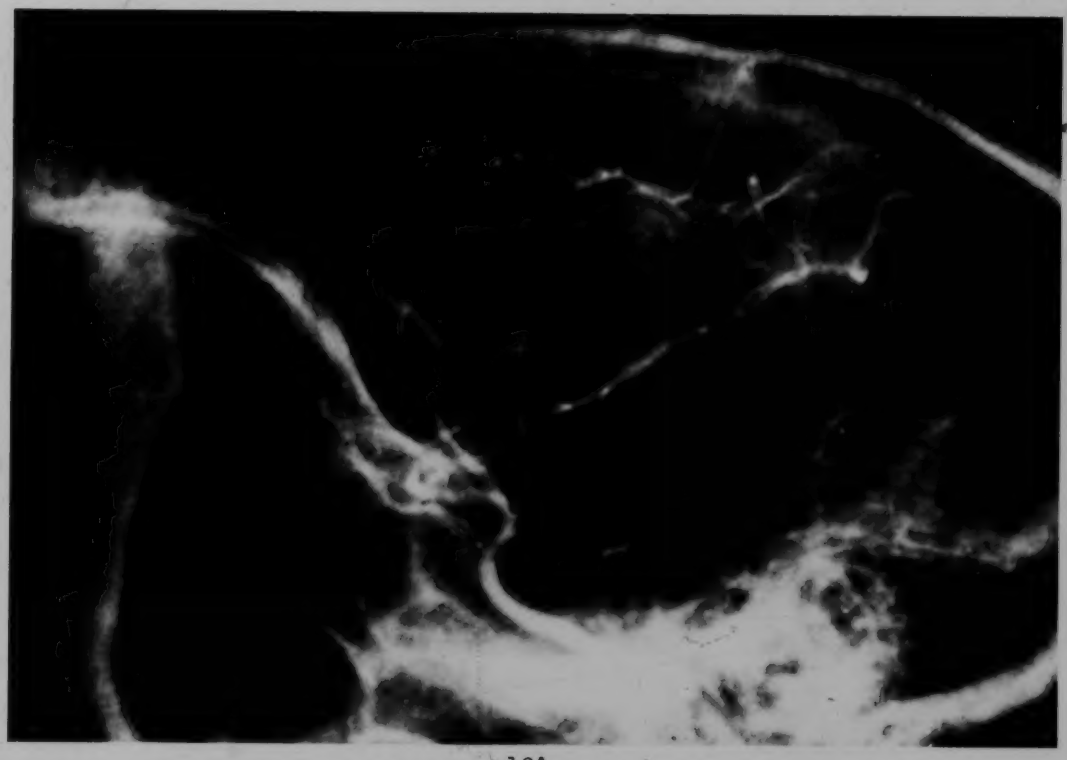
Fig. 18 Regional CBF responses to SAH in one animal illustrating the global nature of flow changes. Probes 1, 2, 3 and 5 measured flow from the frontal, parietal, occipital and temporal regions.

Fig. 19 SAH-induced angiographic cerebral vasospasm

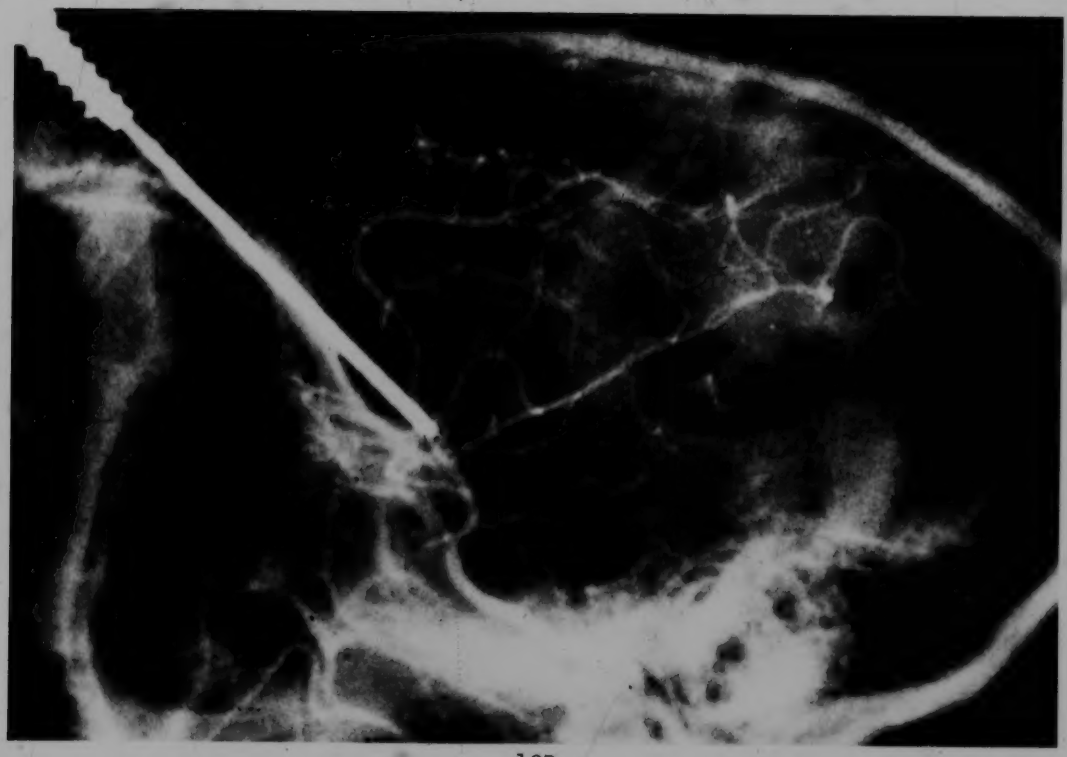
A. Pre-SAH lateral angiogram

B. Post-SAH (1 hr) lateral angiograms showing moderate diffuse vasospasm

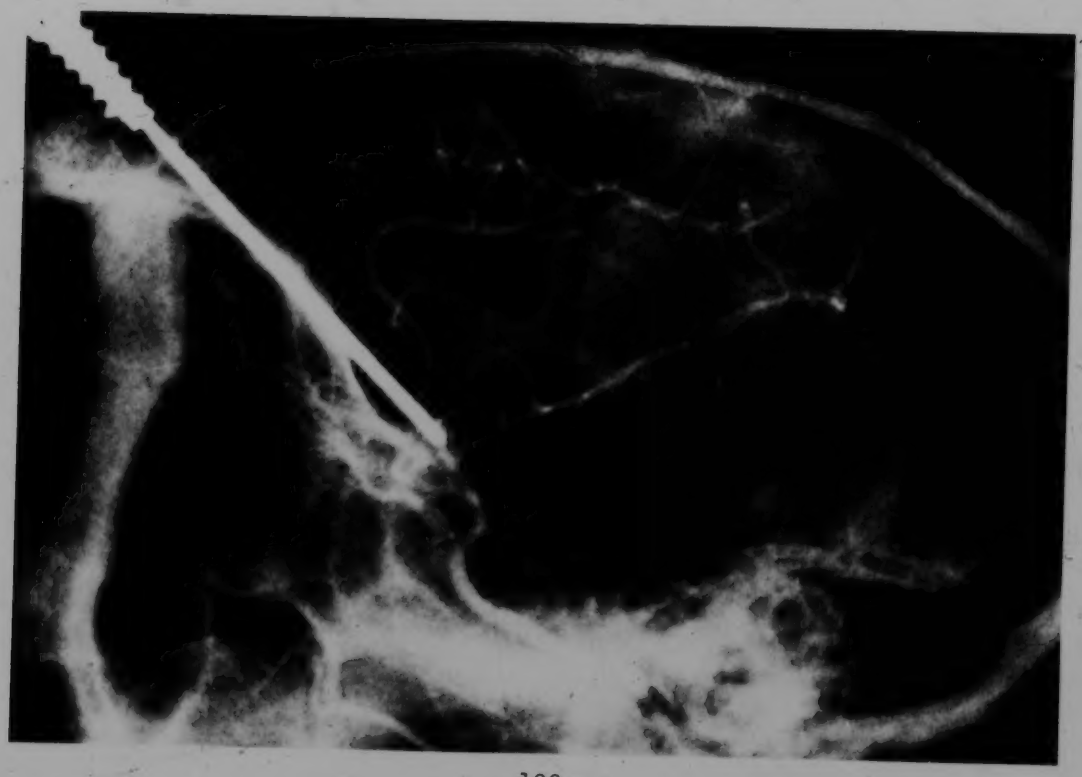
C. Post-SAH (2½ hrs) lateral angiograms. Vasospasm is still present



19A



19B



19C

a decrease in spasm in the 2½ hrs following SAH.

CPP, like CBF, was significantly decreased immediately following SAH suggesting that the transient decrease in CBF observed following SAH may have been partly due to a fall in CPP. An increase in CPP (≈ 15%) was observed in the SAH-CH animals during the latter portion of the experiment. No concurrent change in CBF was observed in these animals. The cause of the increase in CPP was not evident.

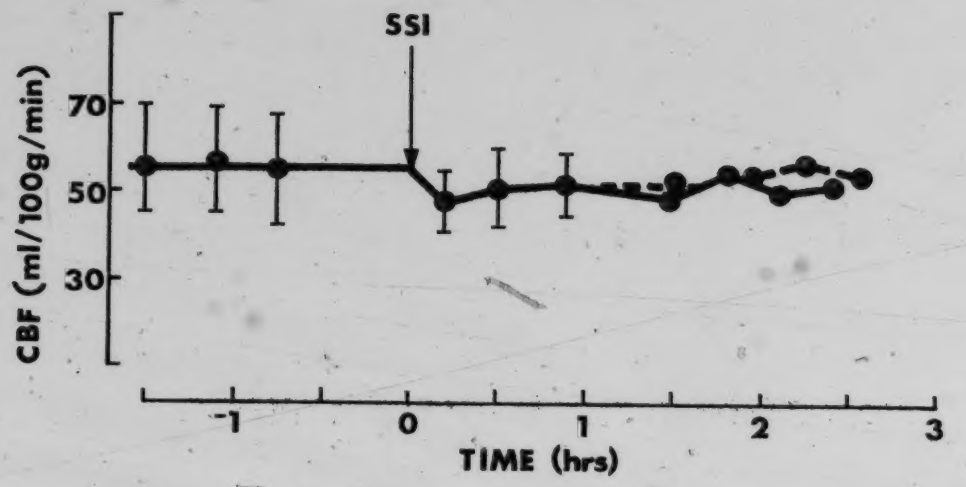
(iii) SSI - Subarachnoid serotonin injection

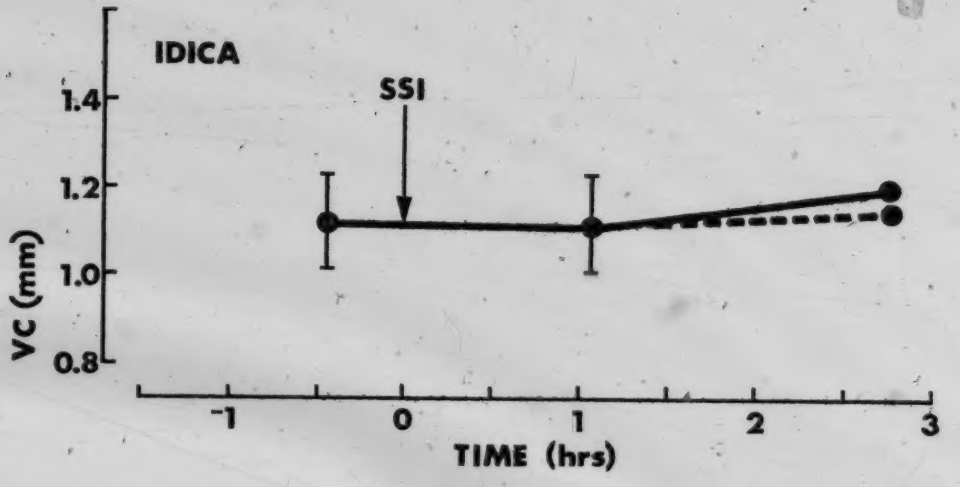
The effects of SSI on CBF (mean hemispheric blood flow), cerebral vessel caliber (IDICA) and CPP are shown in Fig. 20. Subarachnoid injection of serotonin 5×10^{-6} M concentration caused a small nonsignificant decrease in CBF in this group of 10 animals. IDICA caliber and CPP remained unchanged following SSI and following subsequent cyproheptadine administration in 5 of the animals.

A further series of experiments was then carried out in 4 monkeys in which the concentration of the serotonin solution injected into the subarachnoid space was 5×10^{-5} M. The effects of this SSI on CBF (mean hemispheric blood flow), cerebral vessel caliber (MCA) and CPP are shown in Fig. 21. CBF decreased following SSI and was signfiicantly lower than pre-hemorrhage levels at 40 minutes post-SSI. A subsequent gradual recovery of CBF occurred which was complete in 1½ hours.

The results of vessel caliber measurements are shown for the MCA which exhibited the greatest degree of spasm following SSI. Cerebral angiography, performed at 4-5 minutes following SSI, revealed that the spasm occurred almost immediately. Progressive relaxation of this spasm occurred and at 2 to 2½ hrs vessel caliber was not significantly different from the pre-hemorrhage value. Cerebral vasospasm induced by subarachnoid

Fig. 20 Mean and standard deviation of CBF (mean hemispheric blood flow), intradural internal carotid artery (IDICA) caliber and cerebral perfusion pressure (CPP) for 10 animals subjected to subarachnoid injection of a 5×10^{-6} M serotonin solution (3 ml.). Dashed lines connect values for 5 of the 10 animals which received intravenous cyproheptadine (1 mg/kg).


SSI
CBF (ml/100g/min)
70
50
30
-1
0
1
2
3
TIME (hrs)


IDICA
SSI
VC (mm)
1.4
1.2
1.0
0.8
-1
0
1
2
3
TIME (hrs)

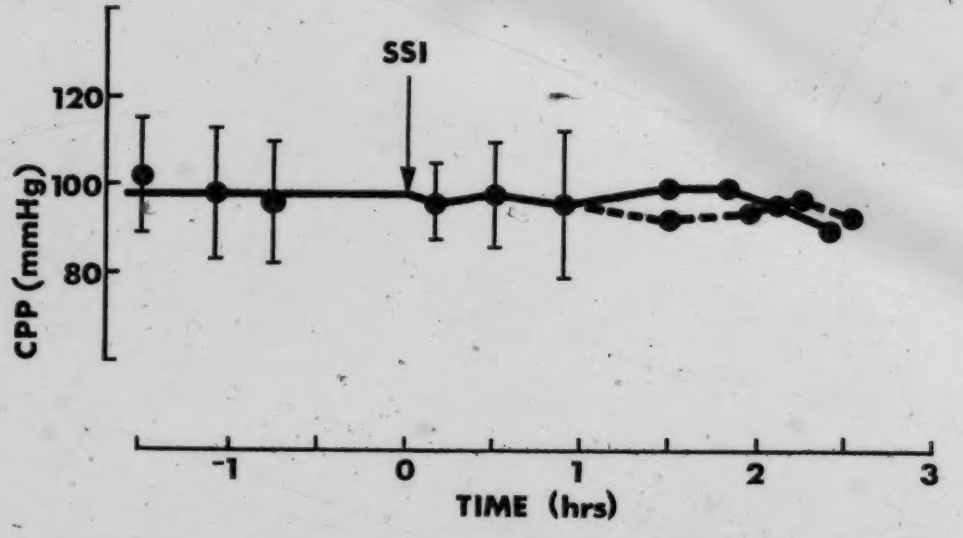
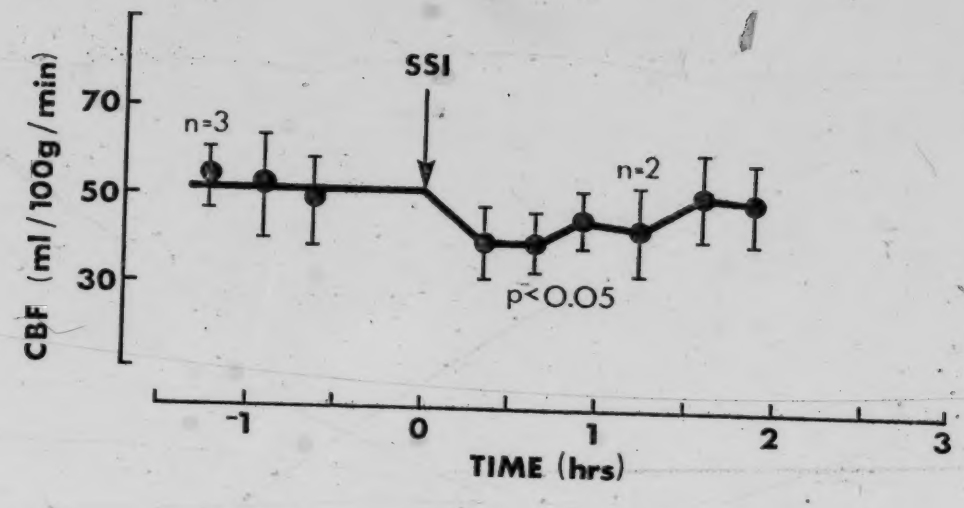
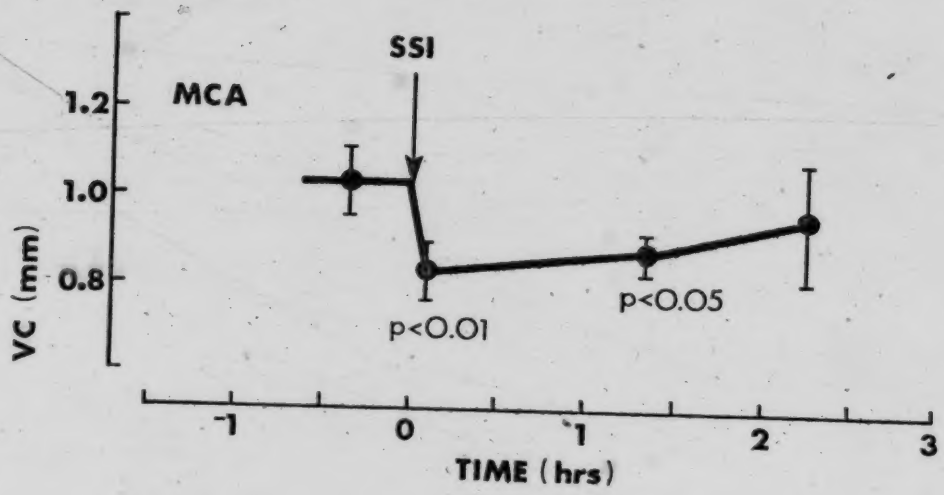
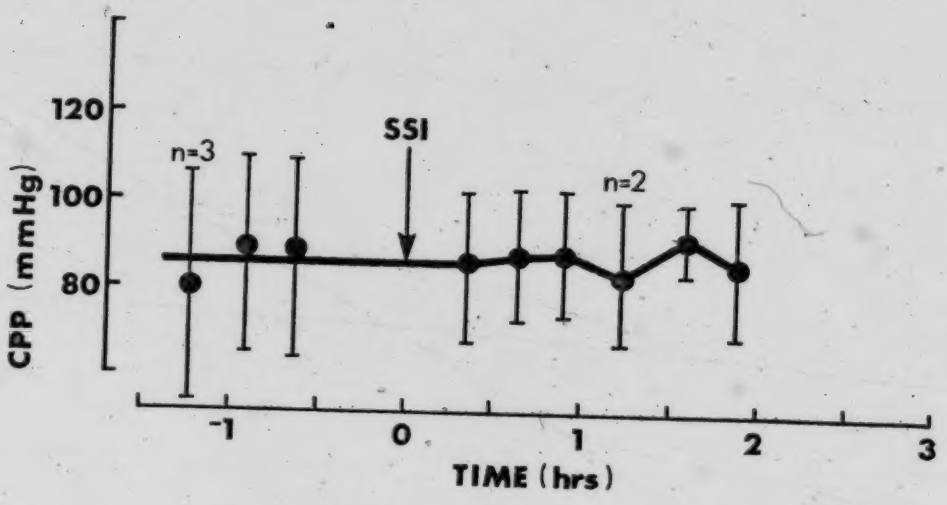

SSI
CPP (mmHg)
120
100
80
-1
0
1
2
3
TIME (hrs)

Fig. 21 Mean and standard deviation of CBF (mean hemispheric blood flow), middle cerebral artery (MCA) caliber and cerebral perfusion pressure (CPP) for 4 animals subjected to subarachnoid injection of a 5 x 10-5 M serotonin solution (3 ml.).


CBF (ml/100g/min)
70
50
30
SSI
n=3
n=2
p<0.05
-1
0
1
2
3
TIME (hrs)


VC (mm)
1.2
1.0
0.8
MCA
SSI
p<0.01
p<0.05
-1
0
1
2
3
TIME (hrs)


CPP (mmHg)
120
100
80
SSI
n=3
n=2
-1
0
1
2
3
TIME (hrs)

injection of serotonin at 5×10^{-5} M, and its recovery, is shown for one animal in Fig. 22. CPP remained fairly constant throughout the duration of these experiments.

(c) Neurological assessment

The animals were neurologically assessed at 2 hrs and at 16-18 hours following reversal of anaesthesia. Results are shown for each type of experiment in Table 5. Animals which had expired prior to neurological assessment were arbitrarily classed as grade V.

(i) MSAH

Eight of the ten animals subjected to a subarachnoid injection of artificial CSF were neurologically normal (grade I or II) when assessed at 2 hrs. One animal had no localizing neurological deficit but was moderately obtunded and was classed as grade III. This animal, however, had a prolapsed rectum which became strangulated during the course of the experiment and this probably contributed to its poor status at 2 hrs and its death at 16 hrs. The brain appeared grossly normal at post-mortem examination.

One animal in the MSAH-CH group which was grade II at 2 hrs subsequently expired due to post-operative hemorrhage from the neck incision. Another animal in this group died within 1½ hours of anaesthetic reversal without apparent cause.

(ii) SAH

The eight animals with SAH which were considered neurologically normal at 2 hrs remained unchanged at 16-18 hrs. The two animals classified as grade IV at two hours died shortly thereafter. Each of these two animals were stable during the experiments and had normal or near-normal CBF and vessel calibers just prior to reversal of anaesthesia. There was

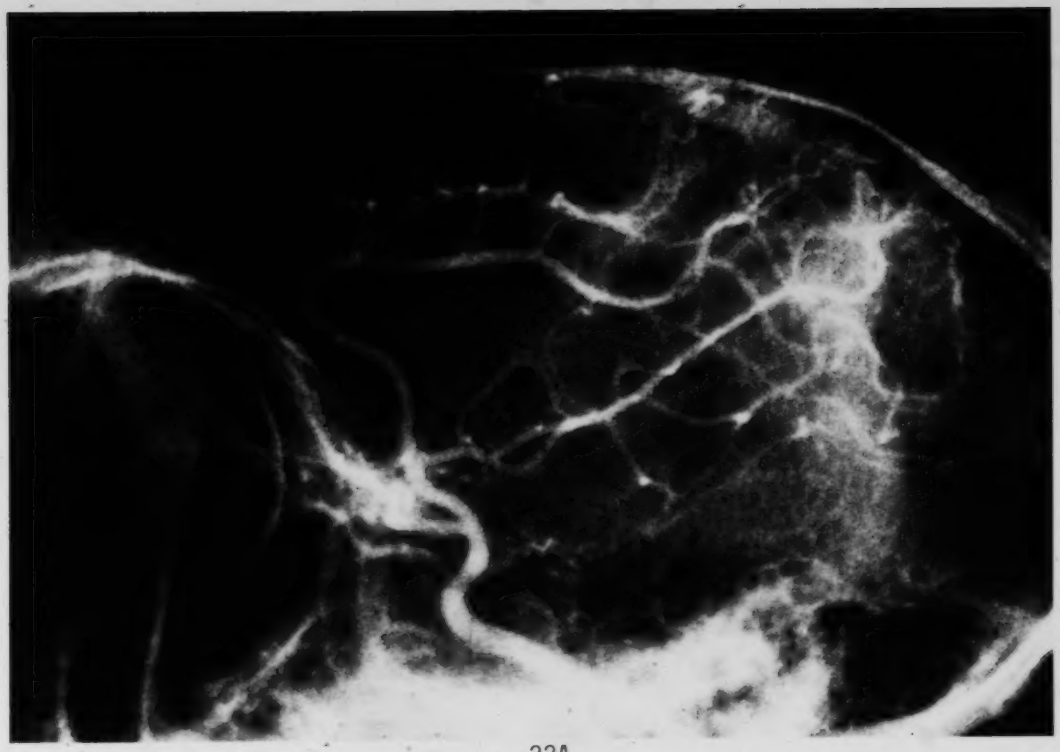
Fig. 22 Serotonin-induced angiographic cerebral vasospasm

A. Pre-SSI lateral cerebral angiogram. Serotonin concentration is 5×10^{-5} M.

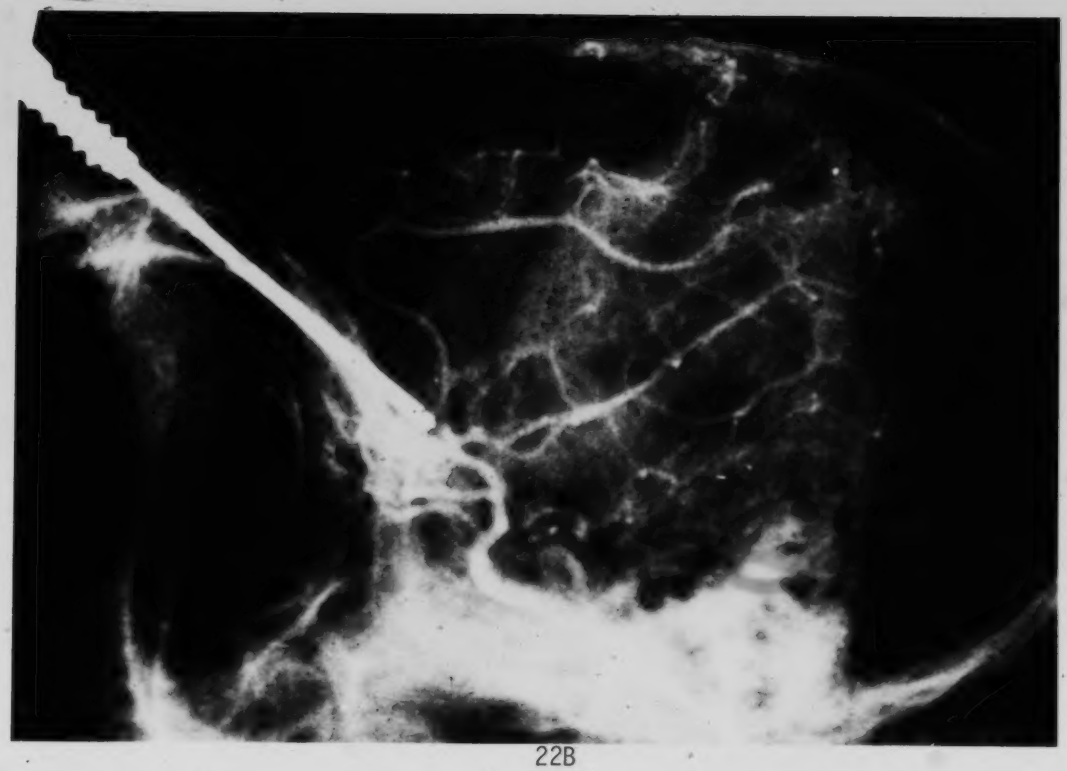
B. Post-SSI (3 min) lateral angiogram showing diffuse vasospasm.

C. Post-SSI (1½ hrs) lateral angiograms

D. Post-SSI (2 hrs 40 min) lateral angiogram. The severity of the vasospasm is decreased.

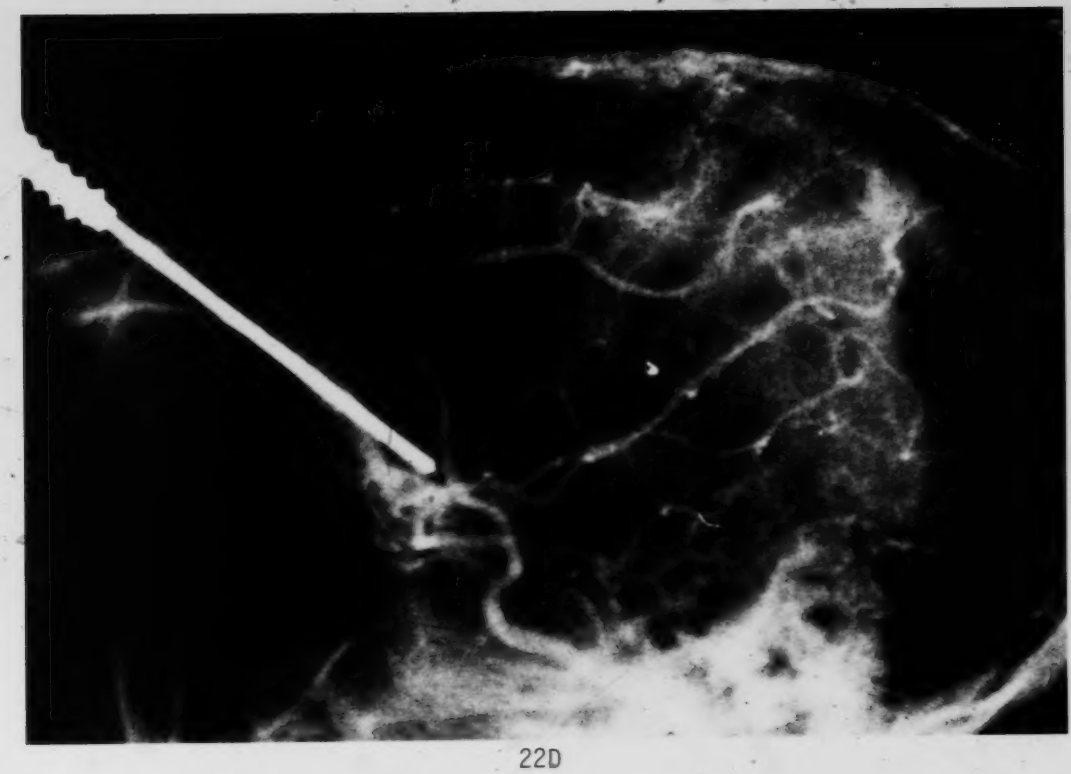


22A



22B

22C



22D

TABLE 5

Expt.	Time	Neurological Grade 1	2	3	4	5	Total
MSAH	2 hrs.	1	3	1	0	0	5
	16-18 hrs.	3	1	0	0	1	5
MSAH	2 hrs.	3	1	0	0	1	5
	16-18 hrs.	3	0	0	0	2	5
SAH	2 hrs.	1	3	0	1	0	5
	16-18 hrs.	4	0	0	0	1	5
SAH-CH	2 hrs.	2	2	0	1	0	5
	16-18 hrs.	3	0	0	0	2	5
SSI ($5x10^{-6}M$)	2 hrs.	2	2	0	0	1	5
	16-18 hrs.	3	1	0	0	1	5
SSI-CH ($5x10^{-6}M$)	2 hrs.	2	2	1	0	0	5
	16-18 hrs.	3	1	0	0	1	5
SSI ($5x10^{-5}M$)	2 hrs.	4	0	0	0	0	4
	16-18 hrs.	4	0	0	0	0	4

Neurological assessment at 2 hrs and 16-18 hrs for each experimental group.

no evidence that their poor neurological status was related to ischemia.

(iii) SSI

One animal with SSI was moderately obtunded with no localizing neurological deficit at 2 hrs and died at 10-12 hrs. The cause of death in this animal was not evident. All other animals with SSI (5×10^{-6} M or 5×10^{-5} M serotonin) were neurologically normal at 2 hrs and at 16-18 hrs.

(D) Cerebral Vessel Ultrastructure

Tissue samples were obtained from animals which survived up to 18 hrs following reversal of anaesthesia (approximately 22 hrs following subarachnoid injection). Of the 18 animals studied, 6 had a SAH, 5 had a MSAH and 7 had a SSI (5×10^{-6} M). Arteries, arterioles and capillaries were examined for evidence of any ultrastructural changes which might be present secondary to cerebral edema or vasospasm.

Cerebral vessel ultrastructure was found to be normal in all animals studied. Particular attention was paid to tissue from animals which demonstrated cerebral vasospasm following SAH; however, these animals also revealed no evidence of cerebral edema or damage to the wall of cerebral arteries from the vasospasm.

Figures 23, 25 and 25 show typical examples of the cerebral vessel ultrastructure of arteries, arterioles and capillaries obtained from animals which demonstrated vasospasm following SAH, and for comparison, from animals subjected to MSAH.

The larger cerebral arteries contained 6-8 layers of smooth muscle in the media (Fig. 23). These smooth muscle cells were morphologically normal in both the SAH and MSAH animals; cell membranes, nuclei and mitochondria were intact. The single continuous layer of endothelial cells lining the arterial lumen showed no evidence of damage in any of

Fig. 23 Arterial ultrastructure

A. Distal pericallosal artery from a MSAH animal

B. Distal pericallosal artery from a SAH animal

Both vessels show normal media (m) and endothelium (e)

H

Fig. 24 Arteriolar ultrastructure

A. Pial arteriole from a MSAH animal

B. Pial arteriole from a SAH animal

In both cases, the media (m) and endothelium (e) are normal in appearance.

A

B

Fig. 25 Capillary ultrastructure

A. Cortical capillary from a MSAH animal

B. Cortical capillary from a SAH animal

In both cases astrocytic processes (a) and surrounding parenchyma are normal in appearance.

A

d

the preparations. Characteristic tight junctions between endothelial cells were always observed. Similar normal structural features were present in small arterioles (Fig. 23) in both SAH and MSAH animals. The presence of muscle cells within the internal elastic lamina (Fig. 23 and Fig. 24) was not an uncommon occurrence. The internal elastic lamina was smooth in all cases, suggesting that these vessels were not in spasm at the time of removal from the brain.

Capillaries in cortical tissue samples (parietal or temporal cortex) from animals with SAH or MSAH were found to have a round, normal appearance with normal tight endothlial connections (Fig. 25). Perivascular astrocytic processes showed no evidence of swelling, nor did adjacent areas of parenchyma.

E. Discussion

(a) In-vitro Studies

Bioassay determination showed that, following clotting, the serotonin concentration of monkey serum was approximately 5×10^{-6} M. This value is similar to the serum serotonin concentration obtained in man (110), baboons (149) and dogs (110). Application of serotonin at 5×10^{-6} M to isolated monkey cerebral vessels produced near maximal contractions in all cases and these contractions were completely blocked by a cyproheptadine concentration of 5×10^{-6} M. This concentration of cyproheptadine was also highly effective in blocking serum induced contractions in the same cerebral vessels. Thus, it appeared that the vasoconstrictive activity of the serum was due to serotonin. Other vasoconstrictive agents which might have been present include norepinephrine and prostaglandins, in particular prostaglandin $F_{2\alpha}$. However, marked contractions of monkey cerebral vessels were produced by $PGF_{2\alpha}$ in the

presence of cyproheptadine. Gilbert and Goldberg (150) have recently demonstrated that cyproheptadine (10^{-6} M) does not alter norepinephrine response in isolated canine carotid and femoral arteries.

These results suggested that the cerebral vasospasm due to blood in the subarachnoid space could be due to the cerebral vasoconstrictor effect of serotonin and that this vasoconstriction could be aborted by cyproheptadine.

(b) In-vivo Studies

Subarachnoid hemorrhage, induced by injection of blood into the prechiasmatic cistern, resulted in a significant but transient decrease in CBF in all regions of the brain; a pattern which has been observed by other investigators under similar experimental conditions (137, 144). Following an initial decrease, CBF returned to normal levels within one hour in-spite of a significant persistent decrease in cerebral vessel caliber. These results are in accord with the findings of other investigations (125, 127, 128) in which CBF was found to correlate poorly with the presence of cerebral vasospasm.

An unexpected finding in these experiments was the lack of effect on rCBF or cerebral vessel caliber of subarachnoid injections of serotonin at 5×10^{-6} M concentration. This concentration of serotonin produced near-maximal contractions of monkey cerebral arteries in-vitro. Although some dilution of the serotonin may have occurred upon injection into the CSF spaces, the volume injected was sufficient to displace essentially all of the CSF in the basal cisterns. These results suggest that cerebral vessels in-vivo do not display the same sensitivity to serotonin as isolated cerebral vessels.

Allen et al (109, 110, 111, 113) have recently performed a compre-

hensive study of the role of serotonin in the genesis of cerebral vasospasm. In their initial in-vitro study (109) in which segments of dog basilar or middle cerebral arteries were utilized, serotonin was the only agent tested which produced a maximal contraction at concentrations (5×10^{-7} M) known to be present in blood. In the present in-vitro study the concentration required for maximal contraction (Cmax) of monkey cerebral arteries was 10 to 15 times greater. Toda and Fujita (151) obtained maximal contractions of dog cerebral arteries with 1×10^{-6} M serotonin; in the same study human cerebral arteries obtained at autopsy were maximally contracted when serotonin concentration reached 5×10^{-6} M. Allen et al (113) also utilized human cerebral arteries and found that the Cmax was 5×10^{-7} M. MOreover, serotonin concentrations of CSF obtained from 2 patients 4 to 17 days after SAH were $1\text{-}2 \times 10^{-7}$ M, while CSF from control patients without SAH had no in-vitro contractile activity. This evidence indicates a major role for serotonin in the production of cerebral vasospasm.

However, the results obtained by Allen et al are difficult to reconcile with the observations of the present study; subarachnoid injections of serotonin 100 times greater than those observed by Allen et al (113) in the CSF of SAH patients with vasospasm produced only transient decreases in CBF and vessel caliber in the monkey. The duration of these changes was 1½ to 2 hours and the magnitude of the decreases, while significant, was not large. Allen et al (109) found that maximal contractions of canine cerebral arteries were obtained with 5×10^{-7} M serotonin. However, in a subsequent study cisternal injections of serotonin in dogs at much higher concentrations (1×10^{-6} to 1×10^{-5} M) produced only 20-30 per cent decreases in the diameter of the basilar arteries (111). These

results, and the similar results obtained in the present study in monkeys, demonstrate that the use of in-vitro preparations of cerebral arteries produces large overestimations of the ability of serotonin to constrict cerebral arteries in the intact animal. Furthermore, it appears that large unphysiological concentrations of serotonin are required to produce significant in-vivo cerebral vasospasm.

Intravenous administration of cyproheptadine failed to relieve the cerebral vasoconstriction which had been induced by SAH. There are two possible explanations for this finding: (1) The vasoconstriction was caused by some agent other than serotonin, or (2) cyproheptadine, when administered intravascularly does not reach the cerebral arterial smooth muscle in sufficient concentrations to have any effect.

Thus, the results of the present study do not allow the formulation of any positive conclusions regarding the role of serotonin in the production of post-SAH cerebral vasospasm in humans. Moreover, it should be emphasized that in this and previous studies the effect of serotonin has been tested on normal cerebral arteries, under normal physiological conditions. Such a state is unlikely to exist in patients with post-SAH cerebral vasospasm.

(c) Electron microscopy studies

The occurrence of ultrastructural changes in cerebral vessels that have been in spasm has been recently reported by Fein et al (152). Cerebral vasospasm was induced in rhesus monkeys by intracisternal injection of blood or puncture of the intradural internal carotid artery. Subsequent electron microscopic examination of cerebral vessels (2 animals) in which severe spasm was observed angiographically revealed changes in smooth muscle cells as early as 8 hrs after onset of spasm. These changes

consisted of condensed lysosomes and degenerating mitochondria. On the light microscopic level these vessels demonstrated a reduction in lumen size and corrugation of the internal elastic lamina. Vessels in which severe spasm persisted for more than 2 days contained necrotic muscle cells, rounded endothelial cells and separation of the normally tight endothelial connections.

In the present study, cerebral arteries and arterioles obtained from monkeys which demonstrated cerebral vasospasm for up to 3 hrs following SAH were examined for ultrastructural changes. These vessels did not demonstrate any ultrastructural abnormalities. This finding may have been due to a number of factors: (1) The vasospasm observed was not severe; (2) since vasospasm was not documented for longer than 3 hrs, the duration of the spasm may have been considerably less at 20 hrs, the time at which the animals were sacrificed. The absence of vasospasm at 20 hrs is indicated by the fact that shrinking and corrugation of the internal elastic lamina was not observed.

Dodson et al (153) performed an ultrastructural study on cerebral tissue obtained from baboons subjected to injection of 10 ml of blood into the subarachnoid space in the region of the circle of Willis. Cortical tissue samples, removed at 24 hrs after SAH, revealed changes typical of cerebral edema i.e. swelling of the pericpaillary astrocytes and widening of the extracellular space. These changes were not observed in the present study; capillaries, astrocytes and surrounding parenchyma were normal in appearance in animals with SAH, MSAH or SSI.

These discrepant results are probably related to differences in the method used to induce SAH. In the animals studied by Dodson et al, 10 ml of blood were injected into the subarachnoid space over a period of 10 minutes. This resulted in a rise in ICP (> 30 mm Hg) which

lasted 12-13 minutes. Such a sustained increase in ICP was sufficient to cause significant cerebral hypoxia as evidenced by the development of cerebral hyperemia for 20 minutes following SAH. In the present study, CSFP was elevated above 30 mm Hg for approximately 2 minutes by subarachnoid injection and no post-injection cerebral hyperemia occurred. Thus, the cerebral edema observed by Dodson et al was likely caused by the relatively prolonged period of cerebral hypoxia which occurred during the induction of SAH.

It would appear then, that the development of post-SAH cerebral edema and intracranial hypertension is dependent upon the duration of the ICP peak which occurs at the time of the SAH. It seems reasonable to assume that the potential for vasospasm-induced cerebral ischemia would be greatly augmented in SAH patients in which the cerebral circulation is already compromised by cerebral edema and intracranial hypertension.

SUMMARY

Studies were carried out in 51 macaque monkeys to further elucidate the effects of subarachnoid hemorrhage on the cerebral circulation. Cerebral perfusion status was assessed directly by regional cerebral blood measurements obtained using the intra-carotid ^{133}Xenon technique, and indirectly by cerebral angiography, neurological assessment and electron microscopic observations of cerebral vessels. Other parameters measured included mean arterial blood pressure, cerebrospinal fluid pressure, heart rate, arterial blood gases and pH, hematocrit, and EKG.

Regional cerebral blood autoregulatory responses to increases in mean arterial blood pressure were measured in 17 adult rhesus monkeys. Efficient autoregulation with essentially constant cerebral blood flow was observed during the pre-hemorrhage period. Angiographic determinations of cerebral arterial caliber responses to increases in mean arterial blood pressure revealed a normal vasoconstrictor response. Subarachnoid hemorrhage was then induced in 12 of these animals; 5 control animals were subjected to a mock subarachnoid hemorrhage by injection of artificial cerebrospinal fluid instead of blood into the subarachnoid space. Following subarachnoid hemorrhage, autoregulation was significantly impaired and was associated with a loss of vasomotor tone. Mock subarachnoid hemorrhage, in contrast, had no effect on autoregulation or cerebral vasomotor tone. No relationship was found between the degree of autoregulatory impairment in the subarachnoid hemorrhage group and neurological status at 1 hr following reversal of anesthetic.

Subarachnoid injections of artificial cerebrospinal fluid, blood, or serotonin were performed in 34 cynmolgous monkeys. Artificial cerebrospinal fluid injections in 10 animals did not affect cerebral blood flow,

arterial caliber or neurological status.

Subarachnoid hemorrhage in a group of 10 animals resulted in a transient decrease in regional cerebral blood flow and moderate vasospasm lasting at least three hours. Administration of the serotonin blocking agent cyproheptadine did not relieve blood induced vasospasm. No significant neurological effect could be attributed to subarachnoid hemorrhage.

Subarachnoid injection of a serotonin solution at physiological concentration in 10 animals had no effect on cerebral blood flow, arterial caliber or neurological status. However, when the concentration of the serotonin solution was increased 10-fold, subarachnoid injection in 4 animals results in a transient decrease in cerebral blood flow similar to that observed with blood. This injection also caused moderate vasospasm; however, the duration of the spasm was shorter than that observed with blood. All 4 animals were neurologically normal following reversal of anesthesia.

Electron microscopic observations failed to reveal any ultrastructural changes in the cerebral vessels irrespective of the type of subarachnoid injection or the presence of vasospasm during the experimental period.

The results of these studies suggest that:

(1) Impairment of cerebral blood flow autoregulation and vasomotor tone occurs following subarachnoid hemorrhage. It is further suggested that this impairment plays an important role in determining the cerebral perfusion status of patients with subarachnoid hemorrhage.

(2) Cerebral vasodilation causing increased intracranial pressure may occur when arterial blood pressure is increased in the presence of impaired cerebral vasomotor tone.

(3) Moderate vasospasm does not impair cerebral perfusion. Angiographic visualization of cerebral arteries does not accurately reflect the cerebral perfusion status.

(4) In-vitro studies overestimate the ability of serotonin to constrict cerebral arteries in intact animals.

(5) Significant vasospasm will not occur unless serotonin is present in high concentrations in the subarachnoid space.

(6) Cerebral edema and intracranial hypertension following subarachnoid hemorrhage is related to the duration of the increase in intracranial pressure which occurs during bleeding from a ruptured aneurysm.

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APPENDIX I

AN AUTOMATIC BOLUS INJECTOR FOR USE IN RADIOTRACER STUDIES OF BLOOD FLOW: DESIGN AND EVALUATION

Early rCBF studies in this laboratory were conducted using the ^{133}Xe intra-arterial injection technique wherein the radioactive bolus was injected by hand. In these studies considerable variation in measured flow values under stable physiological conditions was observed.

An electromechanic device was constructed which automatically injects the ^{133}Xe bolus used in the measurement of rCBF. Results are presented which show that use of the automatic bolus injector in place of hand injection leads to an improvement in the precision of measured flow values. Additional advantages of the device are discussed.

A. MATERIALS AND METHODS

(1) Instrumentation

The main features of the injector system are shown in Fig. 26. Two stainless steel, spring-loaded syringes, one containing the radiotracer solution and the other heparinized saline, are each connected through solenoid operated values* to an outlet manifold and thereby to the arterial catheter system. The approximate volume of the xenon syringe is 12 ml, the saline syringe 37 ml, and of the manifold and catheter (#18 gauge tube 15 cm long) 0.25 ml.

In use, the "xenon valve" (XEV) is programmed to open for a preselected time releasing a small quantity of tracer into the manifold and catheter. Following closure of the xenon valve the "saline flush valve" (SFV) opens for a preselected time flushing residual tracer into the carotid artery.

* ASCO Model 8262B

The electronic control system for the injector is shown schematically in Fig. 27. Following a push-button "inject" command the clock/timer circuits are reset, the XEV armed, and the data acquisition started. After a preset time delay (120 seconds in our experimental studies) during which background measurements are recorded, the XEV is pulsed and a small quantity of tracer introduced into the manifold, catheter and carotid artery. A time delay of 0.1 sec is allowed for the XEV to close, after which the SFV is pulsed and the bolus of tracer flushed into the carotid artery. Regular, short pulsing of the SFV prevents clotting at the catheter tip. This "catheter clear" (volume 0.01 - 0.02 ml) is inhibited during a blood flow measurement. Loading of radioactivity and saline into the syringe system is facilitated by override control (saline fill, xenon fill) of the appropriate solenoid valves.

(2) Experimental Procedure

The method of cerebral blood flow measurement using the ^{133}Xe clearance technique has been described (Chapter 3). In this study, the height/area method of analysis was used to calculate rCBF values for four regions of the brain (Frontal, parietal, occupital and temporal) using the data obtained from four small scintillation detectors. In addition, hemispheric blood flow values were measured using a large, collimated scintillation detector positioned on the contralateral side of the hemisphere being studied. Flow studies were conducted under stable physiolgoical conditions; arterial blood gases, blood pressure, heart rate, EKG and hematocrit were monitored in all animals. All rCBF values obtained were normalized to a $PaCO_2$ of 40 mm Hg (120).

A standard injection bolus of 3.0 mCi of ^{133}Xe dissolved in 0.5 ml of saline was delivered during 1.2 sec, followed by a flush of

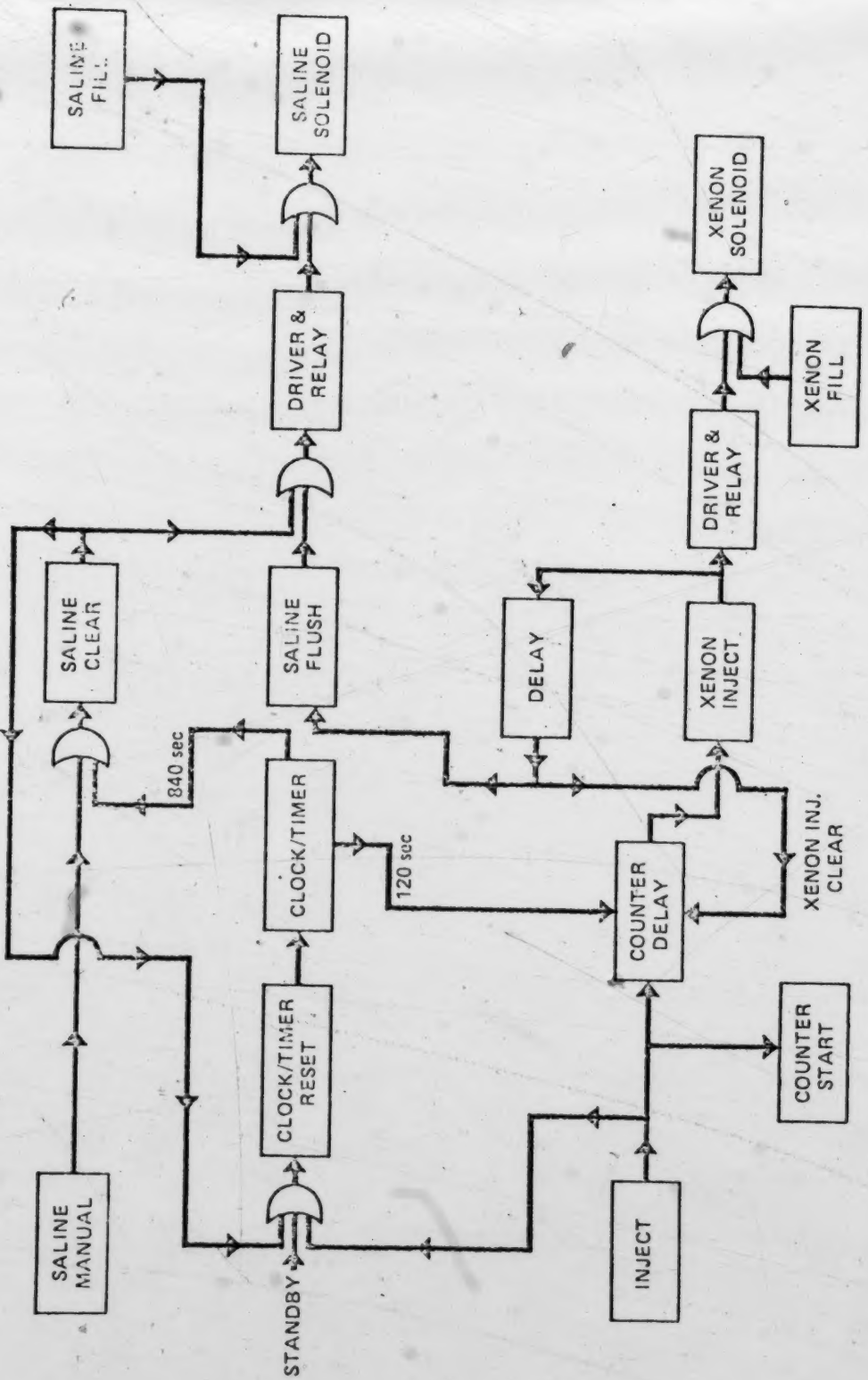

Fig. 27 Injector control unit.

0.8 ml of heparinized saline delivered in 2.7 sec.

Three rhesus monkeys were utilized to determine the effect of changes in injection parameters on measured rCBF. In two animals the effect of decreasing the saline volume by 50% or doubling the ^{133}Xe solution volume (while maintaining the quantity of ^{133}Xe fixed) was studied. The third animal was used to determine the relationship between the quantity of ^{133}Xe injected and rCBF; two similar studies were performed over a five hour period.

The rCBF data obtained from these three animal studies was compared with rCBF data obtained from earlier control experiments in three rhesus monkeys. Although performed by a different investigator, these earlier studies were carried out in a manner identical to the present study except that the arterial injections were performed by hand and no attempt was made to control injection parameters. Reproducibility of rCBF values over a 4-5 hour period was evaluated for each group.

A comparison of hand and automatic injection techniques was carried out with rCBF data from 23 rhesus monkeys used in other experiments. In these animals four consecutive baseline rCBF determinations were made prior to altering physiological parameters. In 11 of the animals ^{133}Xe injections were performed by hand and in 12 animals the automatic injector system was utilized. All flow studies in the 23 animals were performed by this author. Reproducibility of rCBF values over the four baseline flow determinations was evaluated for each injection technique.

B. RESULTS

The effect of altering injection parameters on the measured rCBF values of a single region (parietal)of three rhesus monkeys is shown in Fig. 28. The rCBF values of the remaining regions plus the hemispheric

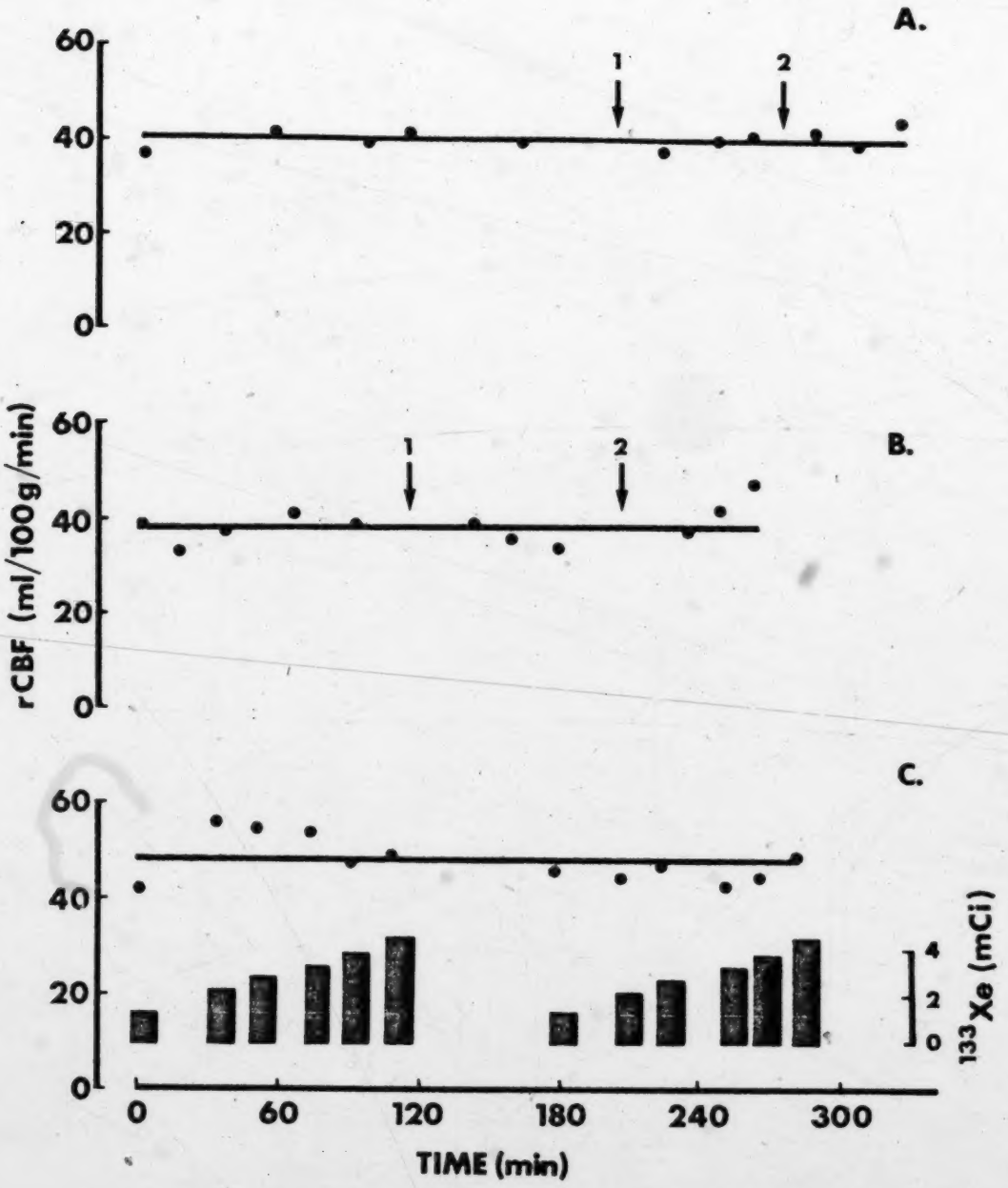

Fig. 28 Cerebral blood flow values for the parietal region in three thesus monkeys. (See text for details). A & B: Arrow 1, flush volume decreased by 50%; arrow 2, flush volume normal, ^{133}Xe solution volume doubled while maintaining injected activity at 3.0 mCi. C: The effect of varying the amount of activity injected on parietal blood flow. Horizontal lines indicate overall mean of parietal blood flow values.

values yielded similar results. Decreasing the saline flush volume by 50% did not produce any significant change in the rCBF values nor did doubling the ^{133}Xe solution volume while maintaining the activity injected at 3.0 mCi. In two studies performed in the same animal the rCBF values remained stabled over a range of injected activity of 1.0 to 4.0 mCi.

The precision of rCBF measurements in earlier control experiments (hand injections) was compared with similar measurements in the three animals used to study the effects of varying injection parameters. A total of twelve hand injection studies (ten flows per study) of rCBF yielded a mean coefficient of variation (percent standard deviation) of 14.7 ± 5.5 (S.D.) with a range of 9.3 - 26.0. Twelve automatic injector studies (eleven or twelve flows per study) yielded a mean coefficient of variation of 9.7 ± 3.3 with a range of 5.1 - 15.2 Thus, a significant improvement ($p < 0.025$) in precision was realized using the automatic injector system. Similar results were obtained in a comparison of the reproducibility in four consecutive baseline flow measurements using both hand (n = 11) and automatic (n = 12) injection techniques. This data is presented in Table 6 where the mean coefficient of variation and its standard deviation are shown for the hand (H) and automatic injection (A) measurements obtained from each of the five (four regional and 1 hemispheric) detectors. It is clear that the mean coefficient of variation is consistently smaller for rCBF measurements using the automatic injector,

C. DISCUSSION

In addition to an improvement in precision for the rCBF measurements, a number of other important benefits are realized using this automatic injector device. For example, following initial connection

TABLE 6

	Frontal	Parietal	Occipital	Temporal	Hemispheric
H	30.9 ± 12.3	25.7 ± 10.2	18.3 ± 9.3	18.8 ± 9.4	18.0 ± 7.8
A	16.8 ± 9.1	11.6 ± 7.7	11.6 ± 10.2	9.4 ± 5.1	9.4 ± 4.4
P	<0.005	<0.001	N.S.	<0.005	<0.005

Mean coefficients of variation ± standard deviation obtained for four regions of the brain and hemisphere using the hand (H) and the automatic injection (A) techniques. Tests for equality of variance were applied to this data and appropriate "t" tests selected for comparative purposes; results are presented in the bottom row.

of the automatic injector manifold to the arterial catheter the fluid filled system remains closed until the completion of the experimental study. This one-time connection decreases the possibility of introducing air emboli into the brain and also greatly reduces the time required to complete a particular experimental study. Experimental duration is also decreased since preparation of individual tracer injections is avoided and because fewer baseline (control) flows are required.

The reduction in handling of the radioactive material significantly reduces the radiation dose to individuals involved. In a hand injection situation where 15-18 measurements of flow are made during the course of an experimental study, a radiation dose of approximately 20 mR is received by the investigator handling the syringes; this dose is reduced to less than 0.5 mR when using the automatic injection device.